AutismCRC

Inflammation and neuromodulation in Autism: Defining an immune-mediated subgroup of children in the Australian Autism Biobank

FINAL REPORT

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The Cooperative Research Centre for Autism (Autism CRC)

The Cooperative Research Centre for Autism (Autism CRC) is the world's first national, cooperative research effort focused on autism. Taking a whole-of-life approach to autism focusing on diagnosis, education and adult life, Autism CRC researchers are working with end-users to provide evidence-based outcomes which can be translated into practical solutions for governments, service providers, education and health professionals, families and people on the autism spectrum.

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A note on terminology

We recognise that when referring to individuals on the autism spectrum, there is no one term that suits all people. In our published material and other work, when speaking of adults we use the terms 'autistic person', 'person on the autism spectrum' or 'person on the spectrum'. The term 'autistic person' uses identity first language, which reflects the belief that being autistic is a core part of a person's identity.

Autism Spectrum Disorder (ASD) is diagnostic terminology used by the healthcare sector, and is used in the context of a person being 'diagnosed with Autism Spectrum Disorder'.

Table of contents

1. Executive Summary 5

1.1. Introduction 5

1.1.1. Diversity on the Autism Spectrum 5

1.1.2. Previous Statistical Approaches to Identifying Subgroups in Autism 5

1.1.3. Evidence for an Immune-Mediated Subgroup in Autism 5

1.2. Research Design and Methods 6

1.2.1. Objectives 6

1.2.2. Methods 6

1.3 Findings 7

1.3.1. Results 7

1.4. Limitations 8

1.5. Implications for Research and Practice 9

1.5.1. Medical and Psychiatric Comorbidity are Important in the context of both Subgrouping Studies and in Clinical Appraisal of Support Needs 9

1.5.2 Cytokine Profiles among Children on the Autism Spectrum in the Australian Autism Biobank differ from Previously Reported Reference Ranges in Non-Autistic Children 10

1.6. Key Recommendations 10

1.6.1. Future Research 10

1.6.2 Clinical Recommendations 10

2. Introduction 11

2.1. Background 11

2.1.1. Empirical approaches to subgroup identification in autistic populations 11

2.1.2. Evidence for an immune-mediated subgroup in autism 12

3. Research Design and Methods 13

3.1. Objectives 13

3.1.1. Primary Objective 13

3.1.2. Secondary Objective 13

3.2. Ethical Governance 13
3.3. Study Sample 13
3.3.1. Participants 13
3.3.2. Assessments 14
3.3.3. Variables 14
3.3.4. Biological Assays 15
3.3.5. Statistical Analyses 15
4. Findings 17
4.1. Cohort Characteristics 17
4.2. Latent Profile Analysis 17
4.2.1. Characteristics of Identified Subgroups 23
4.3. Cytokine Descriptive Results 26
4.3.1. No Significant Subgroup Differences in Cytokine Profiles 28
5. Discussion 29
5.1. Replication and Validation of Subgroup Findings 29
5.2. Cytokine Findings 31
6. Limitations 32
6.1. Need for Further Replication 32
6.2. Factors Influencing Cytokine Concentrations 32
7. Implications for Research and Practice 33
7.1. Medical and Psychiatric Comorbidity are Important in the Context of both Subgrouping Studies and in Clinical Appraisal of Support Needs 33
7.2. Cytokine Profiles among Children on the Autism Spectrum in the Australian Autism Biobank differ from Previously Reported Reference Ranges in Non-Autistic Children 33
8. Key Recommendations 34
8.1. Future Research Recommendations 34
8.2. Clinical Recommendations 34
8.3. Conclusion 35
References 36

1. Executive Summary

1.1. Introduction

1.1.1. Diversity on the Autism Spectrum

Autism is a condition that is widely known to be associated with a large amount of diversity in relation to behavioural traits, associated challenges, co-occurring conditions, and underlying biology. This diversity is referred to as 'heterogeneity.' Heterogeneity poses challenges when studying the usefulness of specific supports or when studying underlying biological processes in autistic populations, because these are believed to vary between subgroups on the spectrum [1].

> **To improve outcomes for children and adults on the autism spectrum, we need to understand which supports work best for different subgroups of autistic individuals. An important first step in this process is the identification of valid and reproducible subgroups in autistic populations.**

1.1.2. Previous Statistical Approaches to Identifying Subgroups in Autism

Over time, emphasis has shifted away from theoretically derived classifications, towards identification of subgroups using data-driven approaches (termed 'empirical methods'). These methods use statistical approaches to identify similarities in patterns of observed data between individuals [2]. The majority of previous studies that have used empirical methods to identify subgroups in autistic populations have mostly focused on data representing the core traits of autism and cognitive intelligence, and sometimes have included data about psychiatric conditions such as anxiety [3]. Co-occurring medical conditions are not often considered in these studies, and many studies have been limited by relatively small sample sizes [4]. Internationally, further research is needed in order to clarify whether specific subgroups can consistently be identified across different autistic populations, and whether the identified subgroups vary in relation to their response to specific supports, and/or their underlying biology.

1.1.3. Evidence for an Immune-Mediated Subgroup in Autism

One specific biological system that may be relevant to autism (or to an autism subgroup) is the inflammatory system. Many previous studies have reported differences between markers of inflammation (such as cytokine profiles) between autistic and non-autistic individuals [5-9].

Cytokines are proteins that serve as markers of inflammation, and can be measured in peripheral blood samples. Cytokines can be classified according to their structure and on the basis of their pro- or anti-inflammatory functions [10]. Many previous studies of inflammatory processes in autism have found higher levels of pro-inflammatory cytokines in autistic individuals compared to non-autistic individuals [5-7, 9, 11-13].

To explore whether there is evidence of an immune-mediated subgroup within children on the autism spectrum in the Australian Autism Biobank (AAB), we performed a latent profile analysis (incorporating data representing the core traits of autism and co-occurring cognitive, medical, and psychiatric profiles), followed by secondary analysis to assess for differences in cytokine profiles in between the identified subgroups. The AAB is a national data repository overseen by the Cooperative Research Centre for Living with Autism (Autism CRC) [14].

1.2. Research Design and Methods

1.2.1. Objectives

The primary objective of this study was to determine whether differing presentations of core traits of autism (pertaining to social communication and to restricted, repetitive, and stereotyped behaviour), in addition to differing cognitive, medical, and psychiatric profiles, could be used to distinguish subgroups of autism using exploratory latent profile analysis of data in the AAB. As a secondary objective, we sought to assess for group differences in cytokine profiles between identified subgroups in the AAB.

1.2.2. Methods

Ethical approval to perform this study was granted by the University of New South Wales Human Research Ethics & Clinical Trials Governance Committee. Data describing behavioural traits and medical history (referred to as ‘phenotypic data’ in this report) were available for all 1151 participants within the AAB, along with access to a subset of 240 biological specimens for immunological assay. This study utilised detailed phenotypic data pertaining to children within the AAB who had received a diagnosis of autism spectrum diagnosis in accordance with DSM-IV or DSM-5 criteria [15], who were recruited between 2013 and 2018 across four sites in Perth, Brisbane, Sydney, and Melbourne. Our subgrouping analysis specifically utilised the data that was available for a subset of 754 children on the AS within the AAB, for whom the deepest phenotypic data (obtained using the Developmental, Dimensional and Diagnostic Interview (3di) [16]) was available. A total of 37 variables were selected for use in our latent profile subgrouping analysis, to

represent core traits of autism, in addition to co-occurring cognitive, behavioural, psychiatric and medical aspects of children's profiles.

1.2.2.1 Biological Analyses of Cytokines

The Australian Autism CRC Utilisation Grant 1.073RU granted this study access to 240 plasma samples obtained from children on the autism spectrum in the AAB, in order for analyses of their cytokine profiles to be performed. These analyses were conducted at Neuroscience Research Australia (NeuRA) using the Magpix Luminex system, using the Bio-plex pro human cytokine 27-plex assay kit (#M500KCAF0Y).

1.2.2.2. Statistical Analyses

Latent profile analysis was used to assess the underlying structure of the phenotypic data within the AAB, by fitting models with increasing numbers of classes (representing subgroups) in a sequential fashion. 'Goodness of fit' statistics were then used to assess which model fit the AAB data best. Thereafter, individuals were allocated subgroup memberships, and differences in their cytokines profiles were examined using multivariate analysis of covariance (MANCOVA), controlling for age-related differences in cytokine profiles.

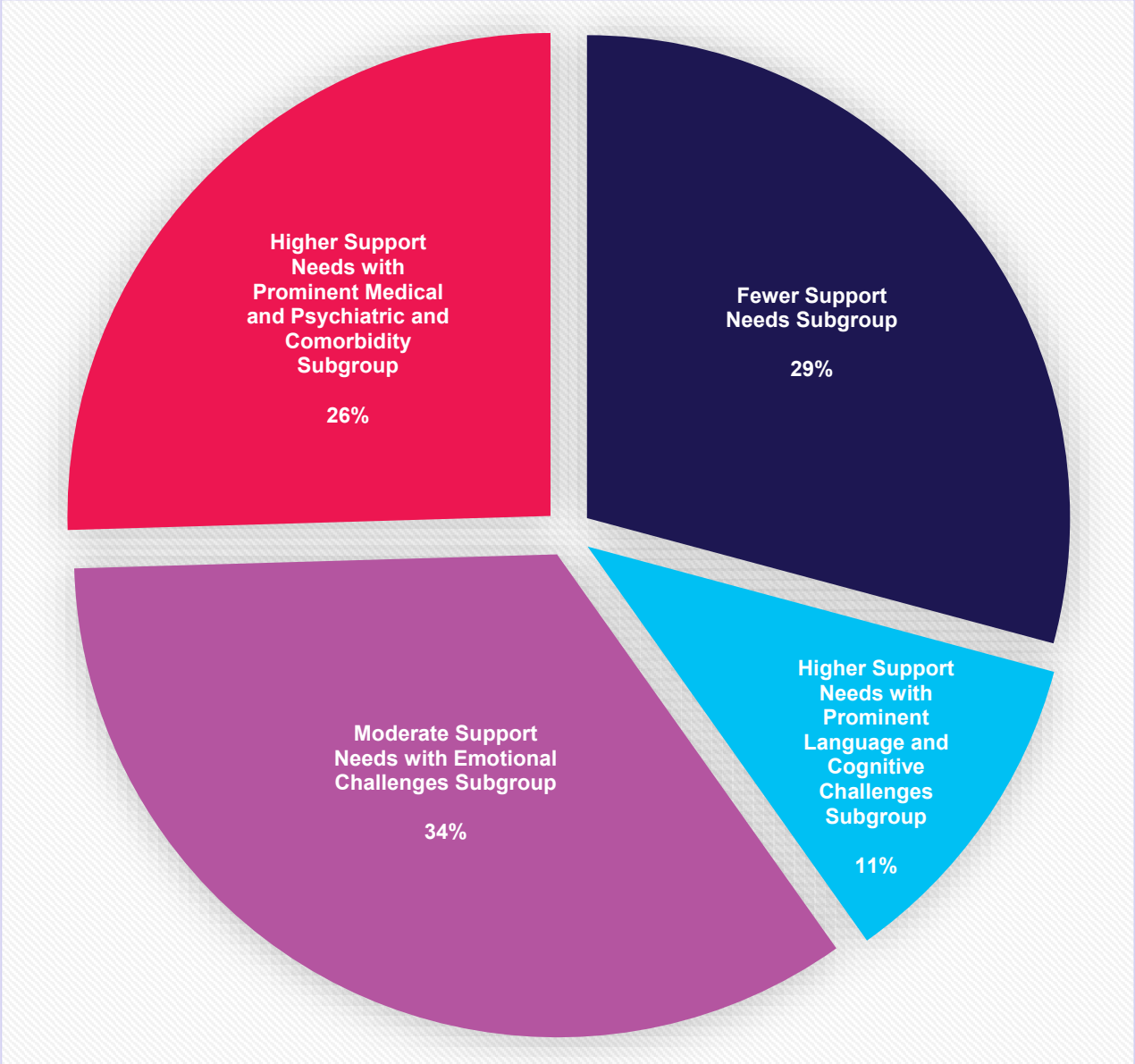
1.3 Findings

1.3.1. Results

Our latent profile analysis found that a four-class model fit the data included in our analysis best. The four subgroups identified are described in Table 1 below. Cytokine profiles did not differ to a statistically significant degree, between the four identified subgroups.

Table 1. Subgroups within the Australian Autism Biobank	
Subgroup One	'Fewer Support Needs Group'
Subgroup Two	'Higher Support Needs with Prominent Language and Cognitive Challenges'
Subgroup Three	'Moderate Support Needs with Emotional Challenges Group'
Subgroup Four	'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity'

Figure 1: Subgroup Membership among Children on the Autism Spectrum in the Australian Autism Biobank



1.4. Limitations

Limited comparison is possible between our findings and those reported in other previous subgrouping studies in autistic populations, because few previous studies have considered medical comorbidity (alongside behavioural, cognitive, and psychiatric data) in their analyses. In those studies where medical comorbidity was considered, differences in the overall range of variables utilised also limits direct comparison with our findings. For these reasons, it is important that future research focus on replication of our findings in other cohorts of children on the autism spectrum, to

validate that the subgroup structure we identified is applicable in a broader context beyond our specific dataset.

A second limitation of note is that interpretation of cytokine findings is complicated by variance in cytokine concentrations associated with numerous factors (e.g. sampling and assay methods, age, gender, genetic, and environmental factors [17]), and we did not have a control sample of non-autistic children to compare our findings to directly in this study.

1.5. Implications for Research and Practice

1.5.1. Medical and Psychiatric Comorbidity are Important in the context of both Subgrouping Studies and in Clinical Appraisal of Support Needs

Our study identified four subgroups of children on the autism spectrum within the AAB that were distinguished not solely on the basis of a 'support needs gradient', but on differing profiles in relation to core autism traits and associated comorbidities. Two subgroups of children had higher support needs compared to the overall group. For the 'Higher Support Needs with Prominent Language and Cognitive Challenges' subgroup, social communication challenges, language delay, cognitive impairment and sensory seeking behaviours were prominent features of the neurodevelopmental profile, but other restricted, repetitive, and stereotyped behaviours (RRBs) were less prominent in this group. The 'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity' subgroup had the highest mean scores of challenges relating to social communication and RRBs, and had the highest probability of medical and psychiatric comorbidity. Interestingly, the 'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity' subgroup had cognitive scores similar to the overall group mean. These findings reflect the importance of considering support needs from a holistic perspective, and validate the inappropriateness of terminology describing individuals as 'high functioning' or 'low functioning,' on the basis of cognitive abilities. Our findings echo those of previous subgrouping studies in autism, where the highest probability of medical and psychiatric comorbidity were observed in subgroups with mean cognitive scores in the average range [2, 18]**.** These findings indicate that cognitive functioning is not a robust indicator of support needs for children on the autism spectrum, and that holistic appraisal of psychiatric and medical comorbidity is essential when characterising the support needs of individuals with neurodevelopmental presentations. To further reiterate this, our findings also indicated that those with moderate mean scores of difficulty associated with core traits of autism had the highest probability of experiencing depression and/or suicidality (the 'Moderate Support Needs with Emotional Challenges' subgroup).

1.5.2 Cytokine Profiles among Children on the Autism Spectrum in the Australian Autism Biobank differ from Previously Reported Reference Ranges in Non-Autistic Children

Our study did not identify significant differences in cytokine profiles between the subgroups of children in the AAB, identified on the basis of behavioural, cognitive, psychiatric, and medical aspects of phenotype. However, our overall mean and median cytokine values differed from those that have been previously reported in non-autistic children in the general population [17, 19, 20]. Our findings are consistent with previous studies that have identified differences in cytokine profiles between autistic and non-autistic control populations [5-7, 9, 11-13]. Further studies are warranted, directly comparing the cytokine profiles of children on the autism spectrum with a control group containing non-autistic children.

1.6. Key Recommendations

1.6.1. Future Research

- Our findings highlight the importance of including co-occurring medical, psychiatric, and cognitive aspects of phenotype among the indicator variables utilised in subgrouping analyses in autistic populations. Future subtyping studies in autism should consider phenotype holistically, and should incorporate variables reflecting medical and psychiatric comorbidity in their analyses where possible.

- Further research is warranted to explore the relevance of immunological differences in children on the autism spectrum.

1.6.2 Clinical Recommendations

- Our findings highlight that clinicians supporting children on the autism spectrum should approach the appraisal of support needs holistically, assessing the impact of co-occurring medical and psychiatric conditions in addition to core autism traits, adaptive functioning, and cognitive functioning.

2. Introduction

2.1. Background

Autism spectrum disorder is a common neurodevelopmental condition characterized by social and communication difficulties in the presence of restricted, repetitive, and stereotyped behaviours [1], with a prevalence of approximately 1% internationally [2]. Clinical, behavioural and biological heterogeneity are widely recognized as hallmark features of the autism spectrum (AS), and this heterogeneity poses a significant impediment to the identification of underlying aetiological processes and targeted treatment and support recommendations [3]. No single etiological pathway is anticipated to be able to explain the majority of the clinical or biological heterogeneity associated with the AS [4]. Rather, a myriad of aetiologies is proposed [5], and the effectiveness of differing treatment approaches will likely vary depending on the putative AS subtypes [6].

2.1.1. Empirical approaches to subgroup identification in autistic populations

There is international consensus that the identification of reproducible, valid subtypes within autistic populations is a priority research area in the context of neurodevelopmental research, to pave the way for identification of genetic and other biomarkers, and targeted treatment and support recommendations for this population [4]. Over time, emphasis has shifted from theoretically derived classifications of subtype to data-driven approaches [7]. A range of confirmatory and exploratory statistical approaches have been utilised for this purpose, such as cluster analysis [5, 8], factor analysis [9], principal components analysis [10] , and latent class or profile analysis [11, 12]. These approaches all seek to identify similarities in patterns of observed data between individuals, and are therefore dependent upon the data variables selected for inclusion in the analysis [7]. The majority of previous studies that have used empirical methods to identify subgroups in autistic populations have classified individuals on the basis of behavioural traits (relating to social communication or RRBs, and occasionally traits indicative of psychiatric comorbidity e.g. anxiety), cognitive or adaptive function, or a combination of behavioural phenotype, cognition and adaptive function [13].

The most replicated findings from empirical studies of subgroup classification in autistic populations to date have yielded between two and four subgroups, defined in terms of support needs (low, moderate, and high) [8, 11-15], and/or two groups endorsing the DSM-5 diagnostic domains (social communication and interaction, and restricted, repetitive, and stereotyped behaviour) [7, 9, 16, 17]. Identified subgroups have not been consistently replicated across

contexts, and have had limited prognostic value to date [18]. However, sample size has been a limiting factor across many previously published studies, requiring that analyses incorporate summary outcome measures as indicator variables (composite scores reflecting categories of behaviour, e.g. total restricted, repetitive, and stereotyped behaviour), rather than measures of specific behaviours reflecting more nuanced phenotypic information. To delve beyond broad diagnostic categories with greater biological and prognostic relevance, constructs that represent specific core traits of autism, in addition to cognitive, medical, and psychiatric comorbidity, must be examined.

Few previous subtyping studies in autistic populations have used both core autism traits and data pertaining to significant comorbidities as indicator variables (such as seizures, gastrointestinal conditions, sleep disorders, and psychiatric conditions) [3], but emerging findings suggest that comorbid conditions (sleep dysfunction, language impairment, immune dysfunction, gastrointestinal dysfunction, and seizures) may be important to discriminating between subgroups within autistic populations [19, 20].

2.1.2. Evidence for an immune-mediated subgroup in autism

Using empirical analytical methods, Sacco and colleagues [10, 19] have previously found immune dysfunction (history of allergy and atopy) to discriminate between subgroups of Italian children on the AS, but this was not replicated in larger samples within the Autism Genetic Resource Exchange (AGRE) and Simons Simplex Collection (SSC) [20]. However, many previous studies have identified inflammatory markers as potential biomarkers in autism [21-25], warranting further investigation to discern whether inflammatory systems are aetiologically relevant in a subgroup of autistic individuals.

Cytokines serve as biomarkers of inflammation, and include families of low-molecular-weight proteins with diverse structure and function, including the interleukins (ILs), interferons (IFNs), tumour necrosis factors (TNFs), and colony-stimulating factors (CSFs) [26]. Cytokines can be broadly classified on the basis of pro- versus anti-inflammatory functions, and on the basis of T helper-1 (Th1) or T helper-2 cell (Th2) mediation of immune responses [27].

Previous studies have identified associations between autism and high levels of pro-inflammatory cytokines including IL-6, TNF-α, GMCSF and IL-8, and with lower levels of anti-inflammatory cytokines such as TGF-β and IL-10 [21-23, 25, 28-30]. Further, autism has been associated with altered function of transcription factors that regulate cytokine and B and T cell receptor expression, such as higher levels of the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB)

both peripherally and centrally [31]. Other associations suggesting that immune dysregulation may play a role in autism include lower levels of TGF-β1, decreased lymphocyte numbers, skewed T helper cells' cytokine profiles, and variations in immune cell and monocyte responses [23, 24].

To explore whether there is evidence suggestive of an immune-mediated subgroup within children in the Australian Autism Biobank (AAB), empirical identification of subgroups (based on core traits of autism and co-occurring cognitive, medical, and psychiatric profiles) will be followed by covariate analyses to explore subgroup differences in cytokine profiles in this study. The AAB is a national data repository overseen by the Cooperative Research Centre for Living with Autism (Autism CRC) [32].

3. Research Design and Methods

3.1. Objectives

3.1.1. Primary Objective

The primary objective of this study was to determine whether differing presentations of core traits of autism (pertaining to social communication and to restricted, repetitive, and stereotyped behaviour), in addition to differing cognitive, medical, and psychiatric profiles, could be used to distinguish subgroups of autism using exploratory latent profile analysis of data in the AAB.

3.1.2. Secondary Objective

As a secondary objective, we sought to assess for group differences in cytokine profiles between empirically identified subgroups, to explore whether evidence to support the existence of an immune-mediated subgroup is identifiable in the AAB.

3.2. Ethical Governance

Ethics to perform this study was granted by the University of New South Wales Human Research Ethics & Clinical Trials Governance Committee (HC190924).

3.3. Study Sample

3.3.1. Participants

Phenotypic data were available for all participants within the AAB (n=1151), along with access to a subset of biological specimens for immunological assay (n=240). The AAB has previously been described in detail by Alvares et al. (2018) [1], and contains detailed phenotypic data and biological samples obtained from children (aged 2-17 years) on the autism spectrum, in addition to siblings, parents, and unrelated non-autistic controls. The empirical subgroup analysis performed in this study utilised detailed phenotypic data pertaining to children within the AAB with an autism spectrum diagnosis in accordance with DSM-IV or DSM-5 criteria [2], who were recruited between 2013 and 2018 across four sites in Perth, Brisbane, Sydney, and Melbourne.

3.3.2. Assessments

Phenotypic data within the AAB was obtained from clinical assessments that utilised a range of administered measures and standardised questionnaires completed by parents or caregivers, including the Autism Diagnostic Observation Schedule-2 (ADOS-2) [3] or Autism Diagnostic Observation Schedule-G (ADOS-G) [4], the Developmental, Dimensional and Diagnostic Interview (3di) [5], Vineland Adaptive Behaviour Scale-II [6], and the Short Sensory Profile-2 (SSP-2) [7]. Cognitive functioning was assessed using the Mullen Scales of Early Learning (MSEL) for those aged below six years [8], or Wechsler Intelligence Scale for Children 4th edition (WISC-IV) for those above 6 years of age [9]. Morphometric measures (height, weight, head circumference), and detailed child and family medical history, were collected for all participants [1]. Data coverage varies across measures, and in this study, latent profile analysis was conducted within the subset of *n*=754 children on the AS within the AAB for whom the deepest phenotypic data (obtained using the 3di standardised parental autism interview) was available. All standardized assessments were administered by raters without knowledge of cytokine measurements.

3.3.3. Variables

In this study, indicator variables pertaining to the core autism traits and psychiatric comorbidity were based on data obtained using the 3di, a standardised parental interview [5]. To reflect aspects of phenotype associated with DSM-5 category A criteria (describing persistent differences in social communication and social interaction), composite-based scores generated by the 3di were used to obtain three continuous measures of difficulty associated with social-emotional reciprocity, non-verbal communication, and development and maintenance of relationships. A further 11 composite-based scores generated by the 3di were used as indicator variables to represent restricted, repetitive, and stereotyped behaviours associated with autism. Appendix A describes the underlying phenotypic constructs and relevant 3di questions contributing to the indicator variables selected to represent core autism traits in this study. Indicator variables selected

to represent aspects of phenotype pertaining to comorbid psychiatric, behavioural, cognitive, and medical conditions were chosen on the basis of existing evidence in the literature for their relevance in relation to autism phenotype [10], and on the basis of their availability in the AAB. Accordingly, 37 indicator variables were selected to represent co-occurring cognitive, behavioural, psychiatric and medical aspects of phenotypes [Appendix A and Appendix B]. Head circumference data were converted to z scores, normed against gender specific population-based samples [11], using Growth Analyser Research Calculation Tools Version 4.1.

3.3.4. Biological Assays

Collection and storage of specimens contained within the AAB has previously been described in detail by Alvares et al. (2018) [1]. Plasma samples were collected between the years 2013 and 2018, and were stored at -80°C. In this study, 100 µL plasma aliquots for each participant were shipped frozen at -80°C to Neuroscience Research Australia (NeuRA) for analysis using the Magpix Luminex system. Cytokines were assayed using the Bio-plex pro human cytokine 27-plex assay kit (#M500KCAF0Y). This kit quantifies a panel of cytokines including MIP-1β, IL-6, IFN-γ, IL-1ra, IL-5, GM-CSF, TNF-α, RANTES, IL-2, IL-1β, Eotaxin, Basic FGF, VEGF, PDGF-BB, IP-10, IL-13, IL-4, MCP-1, IL-8, MIP-1α, IL-10, G-CSF, IL-15, IL-7, IL-12p70, IL-17, and IL-9. Samples were thawed at 4°C for 1 hour, then split into aliquots (3x 30µL, 1x 10µL), one of which was then freeze thawed again, before being prepared according to manufacturer's instructions (#M500KCAF0Y, Biorad). The researcher who ran all assays was blinded to phenotypic details for each participant and a separate researcher assigned samples to individual plates. Plates were processed between 14/4/2021 and 1/6/2021. All samples were run in duplicate wells. Mean inter-plate variability (across analytes) was 17.61% (range 6.98 – 28.8%). 14 plates were processed, all from a single batch (#64373149, plate lot #64301206).

The Australian Autism CRC Utilisation Grant 1.073RU granted this study access to n=240 biological specimens obtained from children on the autism spectrum, for immunological assay (n=240). In keeping with the study budget allocated for these analyses, a total of 215 specimens were processed, due to changes in quoted costs between study design and study execution. Of these 215, one specimen was not contained in the extracted dataset containing phenotypic information from the AAB, and was excluded accordingly.

3.3.5. Statistical Analyses

The distributions of continuous variables were assessed with histograms and bivariate Pearson's or Spearman's correlations were reviewed. The distribution of positively skewed continuous

variables was normalised with logarithmic transformation. Continuous variables were standardised to z scores prior to analysis. Latent class analysis (LCA) and latent profile analysis (LPA) are empirical methods of identifying underlying subgroups (often termed classes) within a dataset based on patterns of data across categorical variables, or continuous variables (or a mixture of both), respectively [1]. In this study, latent profile analysis was conducted using 37 indicator variables, describing 14 core traits of autism [Appendix A], and 23 aspects of phenotype across cognitive, psychiatric, behavioural, medical, and morphometric domains [Appendix B]. The patterns of phenotype represented in our data were assumed to be characterized by an underlying latent categorical variable, and the objective of the analysis was to identify the model that best describes the latent class structure within the dataset, starting with a one-class model and then fitting successive models with increasing numbers of classes. Models are estimated using maximum likelihood techniques, such that there are several solutions around which a model can converge (local maxima). To ensure that a global maximum was identified, we ran at least 200 starts and 20 iterations for each model solution. Goodness of fit statistics was utilised to aid in selection of the optimal model. These statistics included the loglikelihood ratio, with higher values supporting models of better fit, and the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC), with smaller values supporting models of better fit and parsimony [2]. The entropy statistic ranges from 0 to 1, with values closer to 1 reflecting better classification accuracy of individuals into classes depending on their model-based posterior probabilities [3]. Finally, the Lo-Mendell-Runbin Adjusted Likelihood Ratio Test (LMR-LRT) was used to compare models with different numbers of classes, with a non-significant value suggesting that a model with one fewer class is a better explanation of the data [4]. LPA yields predicted probabilities of class membership, and cases were assigned to their most likely class based on these probabilities. Mean scores of continuous indicator variables and differing probabilities for categorical variables were examined by class, in addition to age and gender. Associations between cytokine measures and age were assessed by univariate correlation and differences in cytokine measures by gender were assessed with independent t tests. Differences in cytokine profiles between LPA classes were examined with univariate analysis and with multivariate analysis of covariance (MANCOVA), to determine whether cytokine profiles differed after controlling for age. Assumption testing considered linearity (assessed by visual inspection of scatterplots), univariate outliers (by inspection of boxplots), homogeneity of regression slopes (as assessed by the interaction between age and class), homogeneity of covariances (using Box's M test), and multivariate outliers (using standardised residuals and Mahalanobis distance). Normality of residuals was assessed using Shapiro-Wilk's test (p >.05). Latent profile analysis was performed in Mplus Version 8.6, and all other aspects of the statistical analysis were performed in SPSS Version 26.

4. Findings

4.1. Cohort Characteristics

The overall AAB cohort had a mean age of 7.5 ± 3.9 years, and was predominantly male (78.2%). Deep phenotypic data (obtained from the 3di Developmental, Dimensional and Diagnostic Interview [1]) was available for *n*=754 participants, who were selected for use in the latent profile analysis. These children were similar to the overall AAB cohort, as were the children for whom cytokine profiles were available (*n*=214) [Table 2].

4.2. Latent Profile Analysis

Latent profile analysis of 37 indicator variables describing 14 core traits of autism and 23 other aspects of phenotype yielded a best-fitting model with four-classes. Table 3 shows goodness of fit indices for the latent profile analysis. With each addition of one class to the model, the BIC and adjusted BIC values decreased, but plateaued after the four-class model, whilst the LMR-LRT test suggested that the four-class model did not provide significantly better fit than the three-class model (p = 0.122) [Table 3]. Across models, entropy values were greater than 0.85, suggesting good precision of latent classifications.

Based on goodness of fit statistics, both the three- and four-class models were deemed to best fit the data in this study. The three-class model described three classes differing on the basis of support needs across measures of core autism traits, medical comorbidities, and psychiatric comorbidities. The four-class model was deemed to be more substantively meaningful, describing a 'Fewer Support Needs Group,' 'Higher Support Needs with Prominent Language and Cognitive Challenges Group,' 'Moderate Support Needs with Emotional Challenges Group' and a 'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity Group' [Table 4]. Notable differences between subgroups identified in the 4-class model are summarised in Table 5.

Table 2: Cohort Characteristics

	Australian Autism Biobank Cohort					
	All Children on the Autism Spectrum		Full Phenotypic Data Available[1]		Cytokine Analysis Sub-sample	
N	1151		754		214	
Child Characteristics						
Age in years						
Mean (SD)	7.5 (3.9)		7.5 (3.8)		8.9 (3.8)	
Range	1.9 – 20.9		2.1 – 20.9		2.2 – 20.9	
Missing	0		0		0	
Sex (*n*)						
Male	900	78.2%	595	78.9%	138	64.5%
Female	251	21.8%	159	21.1%	76	35.5%
Maternal Ethnicity (*n*)						
Caucasian	755	65.6%	514	68.2%	170	79.4%
Aboriginal	7	0.6%	6	0.8%	2	0.9%
Asian	94	8.2%	68	9.0%	1	0.5%
Maori/Pacific Islander	11	1.0%	8	1.1%	2	0.9%
Other	74	6.4%	54	7.2%	2	0.9%
Missing	210	18.2%	104	13.8%	37	17.3%
Paternal Ethnicity (*n*)						
Caucasian	763	66.3%	525	69.6%	171	79.9%
Aboriginal	9	0.8%	4	0.5%	2	0.9%
Asian	85	7.4%	58	7.7%	2	0.9%
Maori/Pacific Islander	10	0.9%	8	1.1%	0	0.0%
Other	65	5.6%	45	6.0%	1	0.5%
Missing	219	19.0%	114	15.1%	38	17.8%
Developmental, Dimensional and Diagnostic Interview (3di) Scores of Core Autism Traits (Mean, SD)						
Difficulties with Social-Emotional Reciprocity (Range 0-2)	1.0 (0.3)		1.0 (0.3)		1.0 (0.3)	
Difficulties with Non-verbal Social Communication (Range 0-2)	0.9 (0.3)		0.9 (0.3)		0.9 (0.3)	
Difficulties with Developing and Maintaining Relationships (Range 0-2)	1.0 (0.3)		1.0 (0.3)		1.0 (0.3)	
Stereotyped and repetitive speech (Range 0-45)	15.8 (10.5)		15.8 (10.5)		16.9 (9.0)	
Stereotyped movements (Range 0-9)	3.0 (2.3)		3.0 (2.3)		3.3 (2.5)	
Stereotyped use of objects (Range 0-9)	3.8 (2.5)		3.8 (2.5)		4.3 (2.5)	
Adherence to routines (Range 0-15)	6.1 (4.0)		6.1 (4.0)		8.1 (4.1)	
Ritualised patterns of behaviour (Range 0-15)	6.2 (4.0)		6.2 (4.0)		7.3 (4.0)	
Resistance to change (Range 0-12)	4.4 (3.4)		4.4 (3.4)		5.7 (3.4)	
Restricted and fixated interests	11.0 (6.0)		11.0 (6.0)		12.2 (6.0)	

(Range 0-27)						
Sensory interests (Range 0-15)	5.0 (3.5)		5.0 (3.5)		5.4 (3.4)	
Hyposensitivity to sensory input (Range 0-9)	2.5 (2.3)		2.5 (2.3)		2.8 (2.6)	
Auditory hypersensitivity (Range 0-4)	2.2 (1.6)		2.2 (1.6)		2.4 (1.7)	
Other Sensory Hypersensitivity (Range 0-24)	8.9 (5.9)		8.9 (5.9)		10.7 (6.2)	
Characteristics	**Mean (SD)**		**Mean (SD)**		**Mean (SD)**	
Overall Intellectual Ability (Percentile)	24.0 (28.8)		25.1 (29.0)		30.5 (28.7)	
Head circumference (z score)	-0.4 (1.3)		-0.5 (1.2)		-0.4 (1.3)	
Inattentiveness (Range 0-9)	2.9 (2.3)		2.9 (2.3)		3.5 (2.3)	
Hyperactivity and Impulsivity (Range 0-9)	3.2 (2.4)		3.2 (2.4)		3.7 (2.5)	
Fluency of Speech Score	28.8 (5.2)		28.8 (5.2)		29.5 (4.8)	
Adaptive Composite Score (Percentile)	14.7 (21.8)		15.4 (22.6)		14.1 (22.0)	
Characteristics	***n***	**%**	***n***	**%**	***n***	**%**
Language Delay	468	40.7%	462	61.3%	93	43.5%
Missing data	388	33.7%	4	0.5%	38	17.8%
Gross Motor Delay	246	21.4%	242	32.1%	48	22.4%
Missing data	389	33.8%	5	0.7%	38	17.8%
History of Regression	384	33.4%	259	34.4%	69	32.3%
Missing data	186	16.2%	73	9.7%	14	6.5%
Anxiety Disorder	168	14.6%	168	22.3%	60	28.0%
Missing data	397	34.5%	0	0.0%	42	19.6%
History of Depression and/or Suicidality	73	6.3%	73	9.7%	24	11.2%
Missing data	397	34.5%	0	0.0%	42	19.6%
History of Tics	98	8.5%	98	13.0%	31	14.5%
Missing data	397	34.5%	0	0.0%	42	19.6%
History of Hallucinations	62	5.4%	62	8.2%	21	9.8%
Missing data	498	43.3%	0	0.0%	53	24.8%
Oppositional Defiant or Conduct Disorder	90	7.8%	90	11.9%	32	15.0%
Missing data	397	34.5%	0	0.0%	42	19.6%
History of Self-Injurious Behaviour	64	5.6%	64	8.5%	21	9.8%
Missing data	397	34.5%	0	0.0%	42	19.6%
Birthweight						
Low	94	8.2%	66	8.8%	28	13.1%
Normal	679	59.0%	481	63.8%	134	62.5%
Macrosomic	119	10.3%	83	11.0%	21	9.8%
Missing data	259	22.5%	124	16.4%	31	14.5%
History of seizure(s)	122	10.6%	77	10.2%	32	15.0%
Missing data	172	14.9%	72	9.5%	16	7.5%
Sleep Onset Difficulties	249	21.6%	179	23.7%	71	33.2%
Missing data	175	15.2%	60	8.0%	12	5.6%

Sleep Maintenance Difficulties						
Missing data	140	12.2%	104	13.8%	40	18.7%
	186	16.2%	68	9.0%	14	6.5%
Gastrointestinal Dysfunction						
Missing data	420	36.5%	309	41.0%	106	49.5%
	0	0.0%	0	0.0%	0	0.0%
Food Allergy (Likely IgE mediated)	81	7.0%	72	9.5%	28	13.1%
Missing data	0	0.0%	0	0.0%	0	0.0%
Food Allergy (Non-acute reaction)	163	14.2%	150	19.9%	58	27.1%
Missing data	0	0.0%	0	0.0%	0	0.0%
Non-Food Allergy	199	17.3%	179	23.7%	66	30.8%
Missing data	0	0.0%	0	0.0%	0	0.0%
Hyperextensibility	83	7.2%	83	11.0%	21	9.8%
Missing data	397	34.5%	0	0.0%	42	19.6%

[1]Defined in this study on the basis of available Developmental, Dimensional and Diagnostic Interview (3di) data availability.

Table 3: Latent class fit statistics for children on the autism spectrum in the Australian Autism Biobank

Classes	*Loglikelihood*	*Starts Replicated*	*Free Parameters*	*AIC[a]*	*BIC[b]*	*ABIC[c]*	*LMR-LRT[d] (p)*	*Entropy*
1	-26297.881	200 20	57	52709.76	52973.41	52792.41	N/A	N/A
2	-24904.636	200 20	96	50001.27	50445.31	50140.47	<0.0001	0.90
3	-24561.991	200 20	135	49393.98	50018.41	49589.73	<0.0001	0.87
4	-24303.256	200 20	174	48954.51	49759.33	49206.81	0.1216	0.88
5	-24145.339	200 20	213	48716.68	49701.89	49025.53	0.5549	0.87
6	-23998.863	200 20	252	48501.73	49667.32	48867.12	0.7722	0.87
7	-23870.439	800 80	291	48322.88	49668.87	48744.84	0.4666	0.87

[a] Akaike Information Criterion, [b] Bayesian Information Criterion, [c] Sample Adjusted Bayesian Information Criterion, [d] Lo-Mendell-Rubin Likelihood Ratio Test

Table 4: Characteristics by Latent Class for Children on the Autism Spectrum in the Australian Autism Biobank: FOUR CLASS MODEL

	Class One		Class Two		Class Three		Class Four		Overall	
N	220		83		259		192		754	
Child Characteristics										
Age (y), Mean (SD)	6.5 (3.5)		5.9 (3.2)		8.1 (4.0)		8.4 (3.9)		7.5 (3.8)	
Sex (*n*)										
Male	172	78.2%	72	86.7%	207	79.9%	144	75.0%	595	78.9%
Female	48	21.8%	11	13.3%	52	20.1%	48	25.0%	159	21.1%
Developmental, Dimensional and Diagnostic Interview (3di) Scores of Core Autism Traits (Mean score, SD)										
Difficulties with Social-Emotional Reciprocity (Range 0-2)	0.8 (0.3)		1.4 (0.2)		0.9 (0.2)		1.2 (0.3)		1.0 (0.3)	
Difficulties with Non-verbal Social Communication (Range 0-2)	0.8 (0.3)		1.2 (0.2)		0.8 (0.2)		1.1 (0.3)		0.9 (0.3)	
Difficulties with Developing and Maintaining Relationships (Range 0-2)	0.9 (0.3)		1.3 (0.3)		0.9 (0.3)		1.1 (0.2)		1.0 (0.3)	
Stereotyped and repetitive speech (Range 0-45)	11.4 (9.9)		8.3 (11.8)		17.7 (8.3)		21.4 (9.0)		15.8 (10.5)	
Stereotyped movements (Range 0-9)	1.5 (1.4)		3.7 (2.3)		2.7 (1.9)		4.8 (2.3)		3.0 (2.3)	
Stereotyped use of objects (Range 0-9)	2.0 (1.6)		3.3 (2.1)		3.7 (1.9)		6.3 (2.0)		3.8 (2.5)	
Adherence to routines (Range 0-15)	2.4 (1.7)		3.4 (2.5)		6.6 (2.6)		10.9 (2.6)		6.1 (4.0)	
Ritualised patterns of behaviour (Range 0-15)	3.2 (3.1)		4.9 (3.3)		6.0 (2.6)		10.6 (2.6)		6.2 (4.0)	
Resistance to change (Range 0-12)	1.4 (1.3)		2.5 (2.2)		4.4 (2.2)		8.6 (2.4)		4.4 (3.4)	
Restricted and fixated interests (Range 0-27)	6.2 (4.7)		10.7 (5.6)		11.4 (4.6)		16.0 (5.0)		11.0 (6.0)	
Sensory interests (Range 0-15)	2.5 (2.3)		7.0 (3.1)		4.4 (2.6)		7.7 (3.5)		5.0 (3.5)	
Hyposensitivity to sensory input (Range 0-9)	1.5 (1.6)		2.9 (2.4)		2.2 (2.0)		3.7 (2.8)		2.5 (2.3)	
Auditory hypersensitivity (Range 0-4)	1.6 (1.6)		1.6 (1.5)		2.4 (1.6)		2.9 (1.5)		2.2 (1.6)	
Other Sensory Hypersensitivity (Range 0-24)	4.7 (4.2)		6.8 (4.5)		10.0 (5.4)		13.1 (5.5)		8.9 (5.9)	
Other Continuous Variables (Mean, SD)										
Overall Intellectual Ability (Percentile)	21.9 (28.0)		6.6 (19.9)		31.2 (30.3)		27.3 (27.9)		25.1 (29.0)	
Head circumference (z score)	-0.5 (1.3)		-0.9 (1.3)		-0.3 (1.2)		-0.5 (1.2)		-0.5 (1.2)	

Inattentiveness (Range 0-9)	1.5 (1.8)	3.7 (1.8)	3.0 (2.2)	4.0 (2.3)	2.9 (2.3)
Hyperactivity and Impulsivity (Range 0-9)	1.8 (1.7)	3.2 (1.8)	3.4 (2.4)	4.5 (2.5)	3.2 (2.4)
Fluency of Speech Score	29.0 (5.4)	26.5 (5.0)	29.8 (5.2)	28.2 (4.9)	28.8 (5.2)
Adaptive Composite Score (Percentile)	21.7 (27.1)	2.3 (4.6)	18.8 (22.8)	9.9 (18.0)	15.4 (22.6)
Categorical Variables (Probabilities)					
History of Regression	0.2807	0.6208	0.3058	0.4683	0.344
History of Seizure(s)	0.0702	0.1035	0.1115	0.1638	0.102
Sleep Onset Difficulties	0.1339	0.2162	0.3085	0.3372	0.237
Sleep Maintenance Difficulties	0.0875	0.2281	0.0915	0.2642	0.138
Language Delay	0.5963	0.8936	0.5247	0.6320	0.613
Gross Motor Delay	0.4668	0.4035	0.2038	0.2775	0.321
Gastrointestinal Dysfunction	0.2239	0.4502	0.4391	0.5700	0.410
Food Allergy (Likely IgE mediated)	0.0319	0.0591	0.1135	0.1626	0.095
Food Allergy (Non-acute reaction)	0.0765	0.1857	0.2220	0.3176	0.199
Non-Food Allergy	0.0947	0.1081	0.2843	0.4016	0.237
Anxiety Disorder	0.0199	0.1670	0.2791	0.4110	0.223
Birthweight					
Low	0.0947	0.1594	0.0830	0.1227	0.1048
Normal	0.8343	0.6812	0.7118	0.7975	0.7635
Macrosomic	0.0710	0.1594	0.2052	0.0798	0.1317
Oppositional Defiant or Conduct Disorder	0.0303	0.0171	0.1681	0.2055	0.119
Hyperextensibility	0.0942	0.1284	0.0974	0.1372	0.110
History of Self-Injurious Behaviour	0.0168	0.1514	0.0947	0.1209	0.085
History of Tics	0.0660	0.1288	0.1608	0.1641	0.130
History of Depression and/or Suicidality	0.0615	0.0188	0.1317	0.1274	0.097
History of Hallucinations	0.0271	0.0176	0.0953	0.1905	0.082

4.2.1. Characteristics of Identified Subgroups

In this study, Class 1 (29.2%) described a 'Fewer Support Needs Subgroup,' with fewer social communication difficulties and fewer restricted, repetitive and stereotyped behaviours than the overall group, with higher levels of adaptive functioning. This subgroup was somewhat more likely to have had delayed acquisition of early gross motor milestones than the overall group, but were less likely to have experienced developmental regression, and had lower likelihood of cognitive, psychiatric, and medical comorbidity, compared to the overall group.

Class 2 (11.0%) described a 'Higher Support Needs with Prominent Language and Cognitive Challenges Subgroup,' with the greatest social communication and cognitive difficulties overall. This subgroup had the highest probability of regression, language delay, and self-injurious behaviour. Compared to the overall group, this subgroup had higher mean scores for sensory seeking behaviours, and lower mean scores for all other RRBs (including sensory aversive behaviours, repetitive behaviours, fixations, routine-focused behaviours and insistence on sameness). This subgroup had a similar probability of seizures, gastrointestinal dysfunction, and allergy, compared to the overall group, but had a higher probability of sleep maintenance difficulties.

Class 3 (34.4%) described a 'Moderate Support Needs with Emotional Challenges Subgroup,' that had similar mean scores of core autism traits, cognitive ability, and adaptive functioning, to the overall group. This group had the highest probability of experiencing depression and/or suicidality, and had a higher probability of exhibiting sleep onset difficulties and defiant behaviours than the overall group.

Finally, Class 4 (25.5%) described a 'Higher Support Needs with Prominent Medical and Psychiatric Comorbidity Subgroup.' This subgroup had the highest amount of social communication difficulties and the highest scores of restricted, repetitive and stereotyped behaviours overall. Their mean scores of cognitive ability were similar to the overall group, but with lower levels of adaptive functioning. This subgroup had the highest probabilities of medical comorbidity, sleep dysfunction, and psychiatric comorbidity.

Table 5 – Summary of subgroup differences for Children on the Autism Spectrum in the Australian Autism Biobank based on four-class latent profile modelling

	Class One: **Fewer Support Needs Group**	**Class Two:** **Higher Support Needs with Prominent Language and Cognitive Challenges Group**	**Class Three:** **Moderate Support Needs with Emotional Challenges Group**	**Class Four:** **Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity Group**
Social Communication Difficulties	Mean subgroup levels of difficulty **below** overall group mean scores.	Mean subgroup levels of difficulty **above** overall group mean, and most different from group mean overall.	Mean subgroup levels of difficulty similar to overall group mean.	Mean levels of difficulty **above** overall group mean scores.
Restricted, Repetitive, Stereotyped Behaviours	Average levels of difficulty **below** group mean across all RRB categories.	Repetitive speech, insistence on sameness, ritualistic and routine-focused behaviour subgroup scores **below** overall group mean scores. Sensory seeking and hyposensitivity subgroup scores **above** overall group mean, and sensory sensitivity **below** overall group mean scores.	Subgroup mean similar to overall group mean across all RRB categories.	Mean scores **above** overall group mean scores across all RRB categories.
Cognitive Ability	Subgroup mean similar to overall group mean.	Mean subgroup cognitive ability **below** overall group mean, and most different overall.	Subgroup mean similar to overall group mean.	Subgroup mean similar to overall group mean.
Adaptive Functioning	Subgroup mean **above** overall group mean.	Subgroup mean **below** overall group mean and most different overall.	Subgroup mean similar to overall group mean.	Subgroup mean **below** overall group mean.

Regression	Subgroup probability of regression **lower** than overall group.	**Highest** probability of regression.	Subgroup probability of regression similar to overall group.	Subgroup probability of regression **higher** than overall group.
Language	Subgroup probability of language delay similar to overall group.	**Highest** probability of language delay.	Subgroup probability of language delay similar to overall group.	Subgroup probability of language delay similar to overall group.
Motor	Subgroup probability of motor delay **higher** than overall group.	Subgroup probability of motor delay **higher** than overall group	Subgroup probability of motor delay **lower** than overall group.	Subgroup probability of motor delay similar to overall group.
Medical Comorbidity	Subgroup probability of seizures, gastrointestinal dysfunction, allergy **lower** than overall group	Subgroup probability of seizures, gastrointestinal dysfunction, allergy, similar to overall group.	Subgroup probability of seizures, gastrointestinal dysfunction, allergy similar to overall group.	Subgroup probability of seizures, gastrointestinal dysfunction, allergy **higher** than overall group
Psychiatric Comorbidity	**Lowest** probability of anxiety and ADHD, with probability of depression, defiance, hallucinations, and tics **lower** than in overall group.	**Lowest** probability of defiance and depression, with probability of anxiety **lower** than in overall group but scores of inattention **higher** than in overall group.	**Highest** probability of depression, with probability of defiance **higher** than in overall group.	**Highest** probability of anxiety, ADHD, defiance and hallucinations, with subgroup probability of depression and tics **higher** than in overall group.
Sleep	**Lowest** probability of sleep onset and maintenance difficulties.	Probability of sleep onset difficulties similar to overall group with **higher** probability of sleep maintenance difficulties.	Probability of sleep onset difficulties **higher** but probability of sleep maintenance difficulties **lower** than overall group.	**Highest** probability of sleep onset and maintenance difficulties.
Self-Injurious Behaviour (SIB)	Subgroup probability of SIB **lower** than overall group.	**Highest** probability of SIB.	Subgroup probability of SIB similar to overall group.	Subgroup probability of SIB **higher** than overall group.

4.3. Cytokine Descriptive Results

Cytokine profiles obtained from the overall subsample of children within the AAB (*n*=214) are summarised below [Table 6]. Cytokine profiles by class membership obtained from the 4-class LPA model are summarised in Appendix C.

Table 6: Cytokine Profiles for a Subsample of Children in the Australian Autism Biobank (*n*=214)

	Number (%) Below Detectable Levels[2]	Range (pg/mL)	Median (pg/mL)	Mean (pg/mL)	SD (pg/mL)	Distributions described previously in healthy paediatric controls (pg/mL, unstimulated samples) [1]	Function
MIP-1β	Nil	126.2-342.0	218.2	217.1	28.6	Mean decrease with age, maximum <250, median <120pg/mL [2].	Pro-inflammatory chemoattractant [3].
IFN-γ	Nil	2.1 – 38.6	5.6	6.4	4.1	Age associated increase often replicated [1] but a unimodal peak in children aged 7-17 years compared to younger children and adults has been reported, with range ~100-200, median ~150pg/mL [2].	Pro-inflammatory [5].
IL-1ra	Nil	177.3 - 5204.0	954.4	1272.7	949.0	Mean decrease with age [4], but not consistently replicated. Median (IQR) for 1-6 years: 139.2 (92–185.4) and for 7-17 years: 169.2 (134.7–203.6) [2].	Anti-inflammatory [6].
TNF-α	Nil	13.3 - 90.7	29.9	31.7	10.5	Age associated increase often replicated [1], but a unimodal peak at 13-14 years [4] and higher concentrations in children aged 7-17 years compared to younger children and adults have been reported, with maximum <40pg/mL in children 1-6 years and maximum <65pg/mL in children 7-17 years, with medians ~25 and 30pg/mL respectively [2].	Pro-inflammatory [5].
IL-1β	Nil	0.8-109.7	6.3	10.7	14.3	Less than lower limit of detectability (3.2pg/mL) in all subjects [2].	Pro-inflammatory [9].
Eotaxin	Nil	11.4-143.4	43.5	45.4	19.0	Mean increases with age, with maximum <50pg/mL in children 1-6 years, and <110pg/mL in children 7-17 years [2].	Pro-inflammatory, TH2 upregulation, eosinophilic chemoattractant [10].
Basic FGF	3 (1.4%)	4.4-115.2	21.4	25.3	14.1	No significant influence of age, median (IQR) for 1-6 years: 33.9 (30.8-39.5) and	Anti-inflammatory [11].

						for 7-17 years: 40.1 (35.7-49.3) [2].	
PDGF-BB	2 (1.0%)	29.2-22980.0	993.4	1286.0	1848.9	Unimodal, with higher concentrations in children aged 7-17years compared to younger children and adults, with median ~7000 for children 1-6 years, and median <9000 for children 7-17 years [2].	Primarily anti-inflammatory in relation to wound healing and airways disease, with mitogenic properties [13].
IP-10	Nil	83.6-1383.4	253.4	296.3	188.8	No significant influence of age, median (IQR) for 1-6 years: 674.5 (375.4-795.9) and for 7-17 years: 525.8 (387.8-848.9) [2].	Pro-inflammatory chemoattractant [14].
IL-13	Nil	0.5-23.3	1.4	1.9	2.3	Unimodal, with lowest concentrations in children aged 7-17 years compared to younger children and adults, with range 8-18pg/mL [2].	TH1 anti-inflammatory [5].
IL-4	Nil	1.1-7.0	2.8	2.9	1.1	Unimodal, with higher concentrations in children aged 7-17years compared to younger children and adults, with range 5-12pg/mL [2].	TH1 anti-inflammatory [5].
MCP-1	Nil	2.3 - 56.2	11.7	13.7	8.1	No significant influence of age, median (IQR) for 1-6 years: 35.9 (25.6-62.0) and for 7-17 years: 52.0 (26.5-77.9) [2].	Pro-inflammatory chemoattractant [15].
IL-8	14 (6.5%)	1.3 - 378.4	13.7	27.7	49.0	No significant influence of age, median (IQR) for 1-6 years: 30.9 (23.7-32.0) and for 7-17 years: 32.6 (28.2-39.0) [2].	Pro-inflammatory [5].
MIP-1α	3 (1.4%)	0.6 - 7.0	1.4	1.5	0.7	No significant influence of age, median (IQR) for 1-6 years: 7.3 (6.6-8.1) and for 7-17 years: 7.4 (6.3-8.2) [2].	Pro-inflammatory chemoattractant [3].
IL-10	39 (18.2%)	1.0-12.7	2.6	3.2	1.9	Variable distributions reported [1]. No significant influence of age, median (IQR) for 1-6 years: 11.4 (9.5-12.8) and for 7-17 years: 11.3 (8.9-13.7) [2].	TH1 anti-inflammatory [5].
G-CSF	Nil	13.2-225.1	42.9	49.2	27.9	No significant influence of age, median (IQR) for 1-6 years: 36.2 (30.3-49.9) and for 7-17 years: 43.9 (39.3-54.0) [2].	Both pro- and anti-inflammatory effects. Stimulates neurophilic granulocytes but downregulates IL-1, TNF-α, and IFN-γ [16].
IL-7	Nil	5.4-99.8	12.8	15.3	9.8	No significant influence of age, median (IQR) for 1-6 years: 12.1 (10.3-14.4) and for 7-17 years: 13.6 (10.9-20.0) [2].	Pro-inflammatory [18].

IL-17	Nil	4.3-41.6	14.3	15.3	5.9	Mean increases with age, range 60-180pg/mL [2].	Pro-inflammatory [19].
IL-9	Nil	274.4-806.4	493.5	494.3	74.0	No significant influence of age, median (IQR) for 1-6 years: 17.6 (10.9-26.8) and for 7-17 years: 24.6 (20.2-30.5) [2].	Pro-inflammatory [20].

4.3.1. No Significant Subgroup Differences in Cytokine Profiles

One-way MANCOVA was undertaken to explore whether cytokine levels differed based on class membership among children on the AS, after controlling for age. Gender was not considered a covariate in this analysis, as multinomial probability distributions did not vary significantly by class ($\chi2(3) = 5.057$, $p = .168$), whilst mean age did vary between classes (Welch $F(3,311.543)=16.923$, $p<0.001$).

Cytokine values were transformed on the basis of non-normal distributions across latent classes and due to the presence of genuine outliers (which were not deemed measurement or data errors). Square transformation was used for IL1ra, GCSF and MIP1a (for homogeneity of variances) and all other cytokine values underwent logarithmic transformation (to correct positive skew). IL-6, IL-5, GM-CSF, IL-2, VEGF, IL15, and IL12p70 were excluded as they were deemed poor analytes on the basis of warped calibrated values on quality assurance testing using the Luminex Magpix system and Biorad 27-plex cytokine assay kits. IL-9 was omitted due to multicollinearity with MIP1B, assessed by Pearson's correlation after logarithmic transformation ($r=.930$, $p<0.001$).

There was a linear relationship between each pair of cytokines and between age and each cytokine, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes, as assessed by the interaction term between age and class, ($F(60, 302) = 1.011$, $p = .482$) and homogeneity of covariances, as assessed by Box's M test, ($p > .001$). There were no univariate or multivariate outliers, as assessed by no standardized residuals greater than ± 3 or Mahalanobis distance values greater than a specific cut-off point ($p > .001$), respectively. Following transformation as described above, residuals were approximately normally distributed as assessed by visual inspection of histograms.

There was no statistically significant difference between class allocations associated with the four-class latent profile analysis for the combined cytokines variables after controlling for age, $F(57, 316.884) = 0.792$, $p = 0.857$, Wilks' $\Lambda = .672$, partial $\eta2 = .124$.

Similarly, univariate correlations between cytokines and continuous indicator variables and ANOVAs for categorical variables included in the latent profile analysis were not significant after Bonferroni adjustment for multiple testing.

5. Discussion

5.1. Replication and Validation of Subgroup Findings

Although our identified subgroups did not differ on the basis of cytokine profiles, this exploratory covariate analysis was not intended to be a means of external validation for the subgroups identified in our LPA. However, subgroup differences in overall adaptive functioning (based on the ABC score from the VAB-3) provided external evidence of meaningful clinical differences between the subgroups identified in our study, since adaptive functioning was not used as an indicator variable in our LPA.

Limited comparison is possible between our findings and those reported in other previous subgrouping studies in autistic populations, because few previous studies have considered medical comorbidity (alongside behavioural, cognitive, and psychiatric data) in their analyses. In those studies where medical comorbidity was considered, differences in the overall range of variables utilised also limits direct comparison with our findings. For these reasons, it is important that future research focus on replication of our findings in other cohorts of children on the autism spectrum, to validate that the subgroup structure we identified is applicable in a broader context beyond our specific dataset.

In general, comparison of findings reported between empirical subtyping studies in autistic populations is complicated by significant diversity in the range of variables utilised to construct subgroups. The strengths of this study include our sample size, and the comprehensive range of behavioural, cognitive, medical, and psychiatric variables that were utilised in our subtyping analysis. In a recent systematic review of published subtyping studies in autistic populations, of the 156 identified studies, only 16% had a sample size greater than N=1000 [1]. Studies varied significantly in relation to sample size (ranging between N=17 and N=20658), statistical methods, and indicator variables selected to define subtypes. The median number of variables utilised to conduct subtyping analyses was 20, with 80% of studies including fewer than 20 variables overall. The majority of studies utilised core autism traits to construct subtypes, with only a minority incorporating medical aspects of comorbidity into their analysis. Four previous studies included a

combination of behavioural, cognitive, psychiatric, and medical indicator variables [2-5], and an additional two studies performed empirical subgrouping analysis among children on the autism spectrum using sleep-related [6] or immune-related [7] variables only. Our findings are most amenable to comparison with the four previous studies that utilised behavioural, cognitive, psychiatric, and medical indicator variables for subgrouping analyses, and these are explored in greater detail below.

Wiggins et al. (2017) performed latent class analysis in a similarly sized sample of 707 children on the autism spectrum, and incorporated variables reflecting a similar range of behavioural, cognitive, psychiatric, and medical aspects of phenotype, to those used in this study, (although standardised measures used to reflect these differed) [2]. Four subgroups were identified, including a subgroup characterised by mild language delay with cognitive rigidity, another with mild language and motor delay with dysregulation, another with general developmental delay, and another with significant delay with repetitive motor behaviours [2]. Notable parallels were observed between these previously identified subgroups [2], and those identified in this study. Most notably, both studies identified a subgroup characterised by mean cognitive scores in the average range, with high rates of psychiatric and medical comorbidity including gastrointestinal complaints, sleep dysfunction, and seizures. Both studies identified two subgroups with mild and moderate challenges across most variables, and a subgroup primarily characterised by lowest mean scores of cognitive ability. However, some differences between our findings were also apparent. Although both analyses yielded a subgroup with mild social communication difficulties and comorbidity overall, our study did not replicate associated increased scores of cognitive rigidity in this subgroup, as was observed by Wiggins et al. (2017) [2]. Secondly, the subgroup with the highest degree of cognitive impairment in the study by Wiggins et al. (2017) were at greatest risk of seizures and had high scores for motor mannerisms, whereas in our study the subgroup with the lowest mean cognitive score had low mean scores across all RRBs, with the notable exception of sensory seeking.

More limited comparison is possible between our findings and those reported in other empirical subgrouping studies in autistic populations, even among other studies that examined medical comorbidity, due to differences in the overall range of variables utilised. Veatch et al. (2014) performed hierarchical clustering using variables representing core autism traits, adaptive functioning, age, and head circumference, but did not include other aspects of psychiatric or medical comorbidity in their analysis [3]. Their analysis identified two subgroups characterised by lower and higher severity across measures. As in our study, differing patterns of RRBs were found

to be more useful for discriminating between subgroups than were scores of social communication, and head circumference did not significantly vary between subgroups [3] .

Vargason et al. (2019) performed *k*-means clustering in a cohort of 3,278 children on the AS using 70 variables reflecting a range of behavioural, cognitive, psychiatric, and medical aspects of phenotype [4]. Three subgroups were identified, including one predominantly characterised by high rates of co-occurring psychiatric and medical comorbidity (particularly immune-related conditions and gastrointestinal dysfunction), one predominantly characterised by cognitive delay and highest probability of seizures, and one predominantly characterised by low scores of difficulty across measures [4]. As was observed in our study, the subgroup with highest rates of psychiatric and (non-epileptic) medical comorbidity had mean cognitive abilities similar to the overall group mean [4].

Sacco and colleagues (2012) also used empirical analysis (hierarchical clustering and *k*-means) to identify subgroups in a cohort of 245 children on the AS, and concluded that medical aspects of comorbidity were important in distinguishing between groups [5]. A larger number of variables were reduced to four principal components, describing circadian/sensory dysfunction, immune abnormalities, neurodevelopmental delay, and stereotypy. Subsequent cluster analysis performed using factor scores for these four components identified four subgroups, including one characterised by prominent immune abnormalities accompanied by some circadian and sensory issues, one with prominent circadian and sensory dysfunction, one with prominent stereotypies, and one with prominent cognitive challenges and disruptive behaviour [5]. The subgroup with prominent immune-related dysfunction (e.g. allergy, atopy, autoimmunity) demonstrated the lowest probability of cognitive impairment, with higher probability of obstetric complications and gastrointestinal disturbance, compared to the other subgroups and overall cohort [5].

5.2. Cytokine Findings

Our study did not identify significant differences in cytokine profiles between the subgroups identified on the basis of behavioural, cognitive, psychiatric, and medical aspects of phenotype using LPA. However, the overall group of children from the AAB for whom cytokine analysis was performed (*n*=214*)*, demonstrated differences in their cytokine profiles to those previously described in paediatric control populations [8-10]. In comparison to previously reported paediatric reference ranges (obtained using the same magnetic bead based multiplex Bio-Plex assay from Bio-Rad Laboratories [8]), our study showed elevated median and mean levels of numerous pro-inflammatory cytokines (including IL-1ra, IL-1β, MIP-1β, and IL-9) and lower median and mean levels of numerous anti-inflammatory cytokines (including IL-4, IL-10, PDGF-BB, and IL-13).

Median and mean levels of other pro-inflammatory cytokines (MIP-1α, IFN-γ, IP-10, MCP-1, and IL-17), were relatively low in comparison to previously reported paediatric reference ranges [8]. Differences between previously reported norms and cytokine profiles in our cohort were also observed for IL-5, IL-6, IL-15, VEG-F, GM-CSF, IL-12p70, and RANTES, but on quality assurance assessment these cytokines were deemed poor analytes, and these results have been omitted from this report due to uncertain validity.

Our findings are consistent with previous studies that have identified differences in cytokine profiles between autistic and non-autistic control populations [11-17]. Our study did not clearly demonstrate differences consistent with a pro-inflammatory state, nor did our study did not identify phenotypic associations between cytokine profiles and behavioural, cognitive, medical, or psychiatric characteristics in children on the AS. However, our findings support previously identified evidence for differences between inflammatory profiles observed in non-autistic and autistic populations. Further studies exploring inflammatory mechanisms in autism are warranted.

6. Limitations

6.1. Need for Further Replication

Limited comparison is possible between our findings and those reported in other previous subgrouping studies in autistic populations, because few previous studies have considered medical comorbidity (alongside behavioural, cognitive, and psychiatric data) in their analyses. In those studies where medical comorbidity was considered, differences in the overall range of variables utilised also limits direct comparison with our findings. For these reasons, it is important that future research focus on replication of our findings in other cohorts of children on the autism spectrum, to validate that the subgroup structure we identified is applicable in a broader context beyond our specific dataset.

6.2. Factors Influencing Cytokine Concentrations

A second limitation of note is that interpretation of cytokine findings is complicated by variance in cytokine concentrations associated with numerous factors (e.g. sampling and assay methods, age, gender, genetic, and environmental factors [17]), and we did not have a control sample of non-autistic children to compare our findings to directly in this study.

7. Implications for Research and Practice

7.1. Medical and Psychiatric Comorbidity are Important in the Context of both Subgrouping Studies and in Clinical Appraisal of Support Needs

Our study identified four subgroups of children on the autism spectrum within the AAB that were distinguished not solely on the basis of a 'support needs gradient', but on differing profiles in relation to core autism traits and associated comorbidities. Two subgroups of children had higher support needs compared to the overall group. For the 'Higher Support Needs with Prominent Language and Cognitive Challenges' subgroup, social communication challenges, language delay, cognitive impairment and sensory seeking behaviours were prominent features of the neurodevelopmental profile, but other restricted, repetitive, and stereotyped behaviours (RRBs) were less prominent in this group. The 'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity' subgroup had the highest mean scores of challenges relating to social communication and RRBs, and had the highest probability of medical and psychiatric comorbidity. Interestingly, the 'Higher Support Needs with Prominent Medical and Psychiatric and Comorbidity' subgroup had cognitive scores similar to the overall group mean. These findings reflect the importance of considering support needs from a holistic perspective, and validate the inappropriateness of terminology describing individuals as 'high functioning' or 'low functioning,' on the basis of cognitive abilities. Our findings echo those of previous subgrouping studies in autism, where the highest probability of medical and psychiatric comorbidity were observed in subgroups with mean cognitive scores in the average range [2, 18]**.** These findings indicate that cognitive functioning is not a robust indicator of support needs for children on the autism spectrum, and that holistic appraisal of psychiatric and medical comorbidity is essential when characterising the support needs of individuals with neurodevelopmental presentations. To further reiterate this, our findings also indicated that those with moderate mean scores of difficulty associated with core traits of autism had the highest probability of experiencing depression and/or suicidality (the 'Moderate Support Needs with Emotional Challenges' subgroup).

7.2. Cytokine Profiles among Children on the Autism Spectrum in the Australian Autism Biobank differ from Previously Reported Reference Ranges in Non-Autistic Children

Our study did not identify significant differences in cytokine profiles between the subgroups of children in the AAB, identified on the basis of behavioural, cognitive, psychiatric, and medical

aspects of phenotype. However, our overall mean and median cytokine values differed from those that have been previously reported in non-autistic children in the general population [17, 19, 20]. Our findings are consistent with previous studies that have identified differences in cytokine profiles between autistic and non-autistic control populations [5-7, 9, 11-13]. Further studies are warranted, directly comparing the cytokine profiles of children on the autism spectrum with a control group containing non-autistic children.

8. Key Recommendations

8.1. Future Research Recommendations

- Our findings highlight the importance of including co-occurring medical, psychiatric, and cognitive aspects of phenotype among the indicator variables utilised in subgrouping analyses in autistic populations. Future subtyping studies in autism should consider phenotype holistically, and should incorporate variables reflecting medical and psychiatric comorbidity in their analyses where possible.

- Additional validation methods for subgrouping studies were outlined in the recently proposed framework for subgroup validation, named the SUbtyping Validation Checklist (SUVAC) [1]. Cross-method replication will be explored within the AAB using alternative empirical subtyping methods, in addition to replication using a second dataset (such as the Australian Autism Specific Early Learning and Care Centres dataset). Future opportunities for research will also explore parallel validation of the subgroups we identified, involving use of a second set of indicator variables that reflect similar aspects of phenotype to those used in our initial LPA, to assess whether identified subgroups cluster in a similar substantive manner.

- Further research is warranted to explore the relevance of immunological differences in children on the autism spectrum.

8.2. Clinical Recommendations

- Our findings highlight that clinicians supporting children on the autism spectrum should approach the appraisal of support needs holistically, assessing the impact of co-occurring medical and psychiatric conditions in addition to core autism traits, adaptive functioning, and cognitive functioning.

8.3. Conclusion

Our study identified four subgroups within the AAB that were distinguished not solely on the basis of a 'support needs gradient', but on differing profiles in relation to core autism traits and associated comorbidities. Individuals within subgroups share greater homogeneity in relation to their phenotype presentations than the group overall, and may have greater similarity in terms of shared aetiology and response to treatments. Our findings highlight the importance of including co-occurring medical, psychiatric, and cognitive aspects of phenotype among the indicator variables utilised in subgrouping analyses in autistic populations. Further replication studies are warranted for validation of the subgroups identified in our analysis, including longitudinal follow-up studies to explore stability over time and prognosis. Our study replicated previous findings indicating that autistic individuals exhibit differences in their cytokine profiles compared to non-autistic control populations, but our study did not identify associations between cytokine profiles and any aspect of behavioural, cognitive, medical, or psychiatric phenotype among children on the AS. Further research is warranted to explore whether immunological mechanisms play an aetiological role in a subgroup of children on the AS, but our findings suggest that this subgroup will not be readily identifiable on the basis of their clinical presentation alone.

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Appendix A

Appendix A: Indicator Variables Describing Core Autism Traits based on 3di Data

Indicator Variable	Underlying Phenotypic Construct	Contributing 3di Items (#)
Difficulties with Social-Emotional Reciprocity	Unusual social approach	237,716,717,1073
	Difficulties with age appropriate social behaviour	317,321,322,323,677,728
	Difficulty with back and forth conversation	682,688,705,706,719,744,747, 1142
	Reduced sharing of interests	303
	Reduced sharing of emotions and response to emotion	223,224,304,305,307-310,313,314, 624,625
	Reduced offering to share	297,298,299,300,301
Difficulties with Non-verbal Social Communication	Difficulties reading facial expressions	226,707
	Reduced facial expressiveness	257,258,259-264,708
	Reduced social smiling	249,251,252,711
	Reduced eye contact	248,709,710
	Reduced use and reading of body language	271,272,278,279,280,281,282, 283,284,285,729,737,742
	Reduced imitation	751,752
	Reduced appropriateness of non-verbal interaction	743
	Reduced appropriateness of spontaneous emotions	269,741
Difficulties with Developing and Maintaining Relationships	Difficulty adjusting behaviour to suit social contexts	318,319,320,676,702,703,704, 720,1227
	Reduced imaginative play with peers	361,362,363,364,369,370,371,372
	Reduced cooperative social play	330,331,332,333,360,655
	Difficulties making friends	345,347,349,350,351,352,353,355,713,726,727
Stereotyped and repetitive speech	Repetitive conversation or vocalisation, idiosyncratic language, echolalia, neologism	678,679,680,689-697,748,749,1221
Stereotyped movements	Repetitive movements of the hands, fingers, or body	766,767,982
Stereotyped use of objects	Lining up toys, repetitive ways of viewing video content	755,976,1219

Indicator Variable	Indicator Variable	Indicator Variable
Adherence to routines	Routine focused behaviour, difficulties with transitioning	979,1209,1210,1211,1213
Ritualised patterns of behaviour	Rituals involving actions, verbal rituals, play rituals	340,750,756,1216,1218
Resistance to change	Insistence on sameness	758,1212,1214,1215
Restricted and fixated interests	Preoccupations and unusual interests	721,722,723,754,973,974,975, 1208,1287
Sensory interests	Unusual interests of a tactile, olfactory, oral, or visual nature	757,977,978,1220,1288
Hyposensitivity to sensory input	Decreased sensitivity to pain or temperature	1293,1294,1295
Auditory hypersensitivity	Increased sensitivity to ordinary sounds or music	99,101,103,105,107,109,111
Other Sensory Hypersensitivity	To dietary or tactile textures, taste, visual or olfactory stimuli	1217,1230,1277,1278,1289, 1290,1291,1292

Appendix B

Table 2: Indicator Variables Describing Comorbid Conditions

Domain	Indicator Variable	Data Source	Variable Type
Cognition	**Overall Intellectual Ability** (Percentile)	Overall Composite Score or Full-Scale IQ (FSIQ) from the MSEL[1] or WISC-IV[2]	Continuous
Developmental Language	**Language Delay** Delayed acquisition of language milestones	3di[3]	Dichotomous Categorical
	Articulation	3di[3] CCC[4] subscale A - fluency of speech score	Continuous
Motor	**Gross Motor Delay** Delayed onset of sitting unsupported and/or walking	3di[3]	Dichotomous Categorical
Regression	**Regression** History of loss of physical or language skills	3di[3]	Dichotomous Categorical
Psychiatric and Behavioural	**Inattentiveness** Composite-based score	3di[3]	Continuous
	Hyperactivity and Impulsivity Composite-based score	3di[3]	Continuous
	Anxiety Co-occurring anxiety disorder e.g. generalised anxiety, separation anxiety agoraphobia, phobia, panic	3di[3]	Dichotomous Categorical
	Depression and/or Suicidality Co-occurring depression and/or history of suicidality	3di[3]	Dichotomous Categorical
	Tics History of motor or vocal tics	3di[3]	Dichotomous Categorical
	History of hallucinations Possible or definite visual or auditory hallucinations	3di[3]	Dichotomous Categorical

Domain	Indicator Variable	Data Source	Variable Type
	Oppositional Defiant and/or Conduct Disorder Co-occurring oppositional defiant or conduct disorder	3di[3]	Dichotomous Categorical
	Self-Injurious Behaviour Definite or severe e.g. biting, hair pulling, head banging	3di[3]	Dichotomous Categorical
Medical	**Birthweight Category** Low <2500g Normal 2500-4000g Macrosomia >4000g	Family History Questionnaire – Participant Medical History	Categorical
	Seizures Any previous history of seizures or fits	Family History Questionnaire – Participant Medical History	Dichotomous Categorical
	Sleep Onset Difficulties Requires longer than 20 minutes to falls asleep - Rarely or sometimes, versus usually (5-7 times per week)	Family History Questionnaire – Children's Sleep Habits	Dichotomous Categorical
	Sleep Maintenance Difficulties - Wakes more than once a night - Rarely or sometimes, versus usually (5-7 times per week)	Family History Questionnaire – Children's Sleep Habits	Dichotomous Categorical
	Gastrointestinal Dysfunction History of consulting a health professional in relation to constipation, diarrhoea, reflux, vomiting, or abdominal complaints	Family History Questionnaire – Participant Medical History	Dichotomous Categorical
	Food Allergy (Likely IgE mediated, acute reaction) – e.g. respiratory symptoms, angioedema, vomiting, hives, loss of consciousness	Family History Questionnaire – Participant Medical History	Dichotomous Categorical
	Food Allergy (Likely non-IgE mediated) – e.g. non-acute gastrointestinal dysfunction, irritability or other symptoms	Family History Questionnaire – Participant Medical History	Dichotomous Categorical

Domain	Indicator Variable	*Data Source*	*Variable Type*
Medical	**Non-Food Allergy –** History of reactions to non-food allergens	Family History Questionnaire – Participant Medical History	Dichotomous Categorical
	Hyperextensibility Of finger or thumb joints	3di[3]	Dichotomous Categorical
Morphometric	**Head circumference (z score)**	Clinical Proforma Form	

[1] Mullen Scales of Early Learning (MSEL) [1]

[2] Wechsler Intelligence Scale for Children 4th edition (WISC-IV) [2]

[3] Developmental, Dimensional and Diagnostic Interview (3di) [3]

[4] Children's Communication Checklist – 2nd Edition (CCC-2) [4]

Appendix C

Appendix C: Cytokine Profiles by Class Membership Obtained from 4-class LPA Model

	Class One			Class Two			Class Three			Class Four		
	n	Range (pg/mL)	Mean (SD) pg/mL	n	Range (pg/mL)	Mean (SD) pg/mL	n	Range (pg/mL)	Mean (SD) pg/mL	n	Range (pg/mL)	Mean (SD) pg/mL
MIP-1β	31	143.7-334.0	216.1 (34.2)	10	149.1-226.9	208.2 (23.1)	62	158.7-274.5	217.0 (25.5)	69	126.2-263.1	215.6 (27.2)
IFN-γ	31	2.2-13.2	6.0 (3.0)	10	2.7-14.7	6.9 (3.5)	62	2.9-17.4	6.3 (2.7)	69	2.1-38.6	6.8 (5.7)
IL-1ra	31	190.1-4500.0	1433.4 (1110.5)	10	188.9-3166.8	1135.5 (1016.6)	62	211.4-3864.8	1276.5 (831.6)	69	190.1-4572.0	1279.2 (964.1)
TNF-α	31	13.3-90.7	32.6 (14.2)	10	20.8-38.5	31.1 (6.2)	62	17.3-58.7	32.6 (10.3)	69	15.6-62.1	31.1 (9.1)
IL-1β	31	1.1-24.8	9.1 (6.4)	10	1.0-24.0	9.5 (8.4)	62	1.2-109.7	13.3 (21.2)	69	0.8-61.0	10.5 (12.7)
Eotaxin	31	15.2-143.4	46.5 (24.8)	10	23.7-65.0	39.0 (13.0)	62	17.3-108.4	44.8 (17.7)	69	11.4-91.4	45.5 (17.4)
Basic FGF	31	4.4-42.3	22.7 (9.3)	9	7.6-38.9	24.8 (10.5)	62	6.4-115.2	26.5 (19.5)	68	10.6-71.1	26.0 (12.8)
PDGF-BB	31	222.8-11272.0	1412.1 (2041.7)	9	82.2-2763.0	1083.5 (800.4)	62	119.4-2965.7	1077.9 (680.4)	69	29.2-3485.2	1052.1 (764.4)
IP-10	31	133.0-1080.4	298.3 (229.7)	10	86.0-334.8	244.4 (87.9)	62	83.6-1013.1	293.4 (168.3)	69	108.8-1383.4	278.6 (190.5)
IL-13	31	0.7-23.3	2.3 (4.0)	10	0.7-2.4	1.4 (0.5)	62	0.6-6.0	1.6 (0.9)	69	0.5-22.0	2.1 (2.7)
IL-4	31	1.1-7.0	3.0 (1.3)	10	1.6-4.1	2.8 (0.8)	62	1.6-6.3	3.0 (1.1)	69	1.2-5.6	3.0 (0.9)
MCP-1	31	2.3-35.3	13.6 (8.5)	10	4.4-20.2	11.3 (5.7)	62	3.3-42.8	13.3 (7.6)	69	3.3-38.8	14.6 (7.8)
IL-8	29	1.6-263.9	30.6 (50.9)	9	2.3-27.1	12.7 (8.6)	60	2.2-368.9	28.9 (49.6)	63	1.3-378.4	25.5 (53.3)
MIP-1α	31	0.6-3.2	1.4 (0.5)	10	0.7-3.9	1.6 (0.9)	60	0.6-7.0	1.6 (0.9)	68	0.6-6.3	1.5 (0.7)
IL-10	23	1.2-10.1	3.8 (2.5)	6	1.7-8.0	4.4 (2.7)	57	1.0-8.6	3.1 (1.8)	54	1.2-12.7	3.0 (2.0)
G-CSF	31	13.2-108.1	47.7 (23.9)	10	18.3-218.7	57.8 58.3)	62	18.8-210.2	49.0 (28.0)	69	17.6-225.1	47.9 (25.7)
IL-7	31	8.0-66.8	15.5 (10.6)	10	6.8-37.1	17.2 (10.5)	62	5.4-57.6	15.0 (8.3)	69	6.5-99.8	16.4 (12.4)
IL-17	31	4.6-27.6	14.6 (4.8)	10	6.4-20.6	14.3 (4.5)	62	6.8-41.6	16.3 (7.3)	69	4.3-28.5	15.5 (5.2)
IL-9	31	280.9-723.0	491.3 (75.3)	10	341.9-530.3	467.7 (59.4)	62	347.3-627.0	491.0 (69.8)	69	274.4-674.3	492.8 (71.0)

AutismCRC

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Our values

Inclusion

Working together with those with the lived experience of autism in all we do

Innovation

New solutions for long term challenges

Evidence

Guided by evidence-based research and peer review

Independence

Maintaining autonomy and integrity

Cooperation

Bringing benefits to our partners; capturing opportunities they cannot capture alone

Australian Government
Department of Industry, Science, Energy and Resources

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Cooperative Research Centres Program