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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'.

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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 a subgroup with autism) 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.

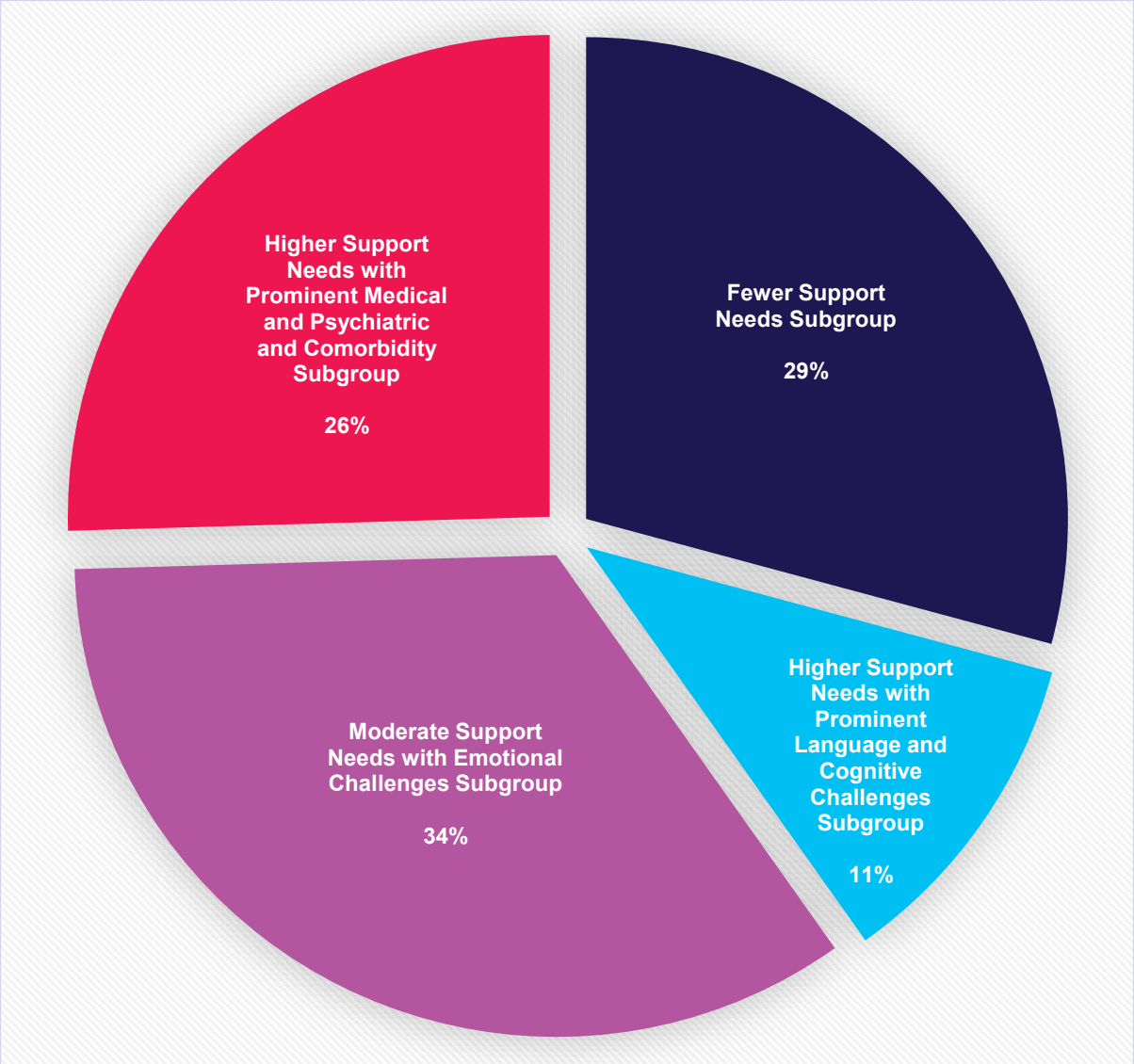
1.3 Findings

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.

1.7. References

1. Masi A, DeMayo MM, Glozier N, Guastella AJ: *An Overview of Autism Spectrum Disorder, Heterogeneity and Treatment Options.* Neuroscience bulletin 2017, 33(2):183-193.
2. Wiggins L, Tian L, Levy S, Rice C, Lee L-C, Schieve L, Pandey J, Daniels J, Blaskey L, Hepburn S et al: *Homogeneous Subgroups of Young Children with Autism Improve Phenotypic Characterization in the Study to Explore Early Development.* Journal of Autism & Developmental Disorders 2017, 47(11):3634-3645.
3. Beglinger LJ, Smith TH: *A review of subtyping in autism and proposed dimensional classification model.* J Autism Dev Disord 2001, 31(4):411-422.
4. Agelink van Rentergem JA, Deserno MK, Geurts HM: *Validation strategies for subtypes in psychiatry: A systematic review of research on autism spectrum disorder.* Clinical Psychology Review 2021, 87:102033.
5. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J: *Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders.* J Neuroimmunol 2011, 232(1-2):196-199.
6. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M: *Activation of the inflammatory response system in autism.* Neuropsychobiology 2002, 45(1):1-6.
7. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, Altaye M, Wills-Karp M: *Elevated cytokine levels in children with autism spectrum disorder.* J Neuroimmunol 2006, 172(1-2):198-205.
8. Enstrom AM, Onore CE, Van de Water JA, Ashwood P: *Differential monocyte responses to TLR ligands in children with autism spectrum disorders.* Brain Behav Immun 2010, 24(1):64-71.
9. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M: *Elevated immune response in the brain of autistic patients.* J Neuroimmunol 2009, 207(1-2):111-116.
10. Martins TB, Rose JW, Jaskowski TD, Wilson AR, Husebye D, Seraj HS, Hill HR: *Analysis of proinflammatory and anti-inflammatory cytokine serum concentrations in patients with multiple sclerosis by using a multiplexed immunoassay.* Am J Clin Pathol 2011, 136(5):696-704.
11. Ashwood P, Anthony A, Pellicer AA, Torrente F, Walker-Smith JA, Wakefield AJ: *Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology.* J Clin Immunol 2003, 23(6):504-517.
12. Ashwood P, Anthony A, Torrente F, Wakefield AJ: *Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10.* J Clin Immunol 2004, 24(6):664-673.
13. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, Ozonoff S, Pessah IN, Van de Water J: *Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes.* J Neuroimmunol 2008, 204(1-2):149-153.
14. Alvares GA, Dawson PA, Dissanayake C, Eapen V, Gratten J, Grove R, Henders A, Heussler H, Lawson L, Masi A et al: *Study protocol for the Australian autism biobank: an international resource to advance autism discovery research.* BMC Pediatrics 2018, 18(1):284.
15. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.* Arlington, VA: American Psychiatric Association; 2013.
16. Skuse D, Warrington R, Bishop D, Chowdhury U, Lau J, Mandy W, Place M: *The developmental, dimensional and diagnostic interview (3di): a novel computerized assessment for autism spectrum disorders.* Journal of the American Academy of Child and Adolescent Psychiatry 2004, 43(5):548-558.
17. Decker ML, Grobusch MP, Ritz N: *Influence of Age and Other Factors on Cytokine Expression Profiles in Healthy Children-A Systematic Review.* Front Pediatr 2017, 5:255.
18. Vargason T, Frye RE, McGuinness DL, Hahn J: *Clustering of co-occurring conditions in autism spectrum disorder during early childhood: A retrospective analysis of medical claims data.* Autism Res 2019, 12(8):1272-1285.
19. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G: *Cytokine levels in the serum of healthy subjects.* Mediators Inflamm 2013, 2013:434010.
20. Sack U, Burkhardt U, Borte M, Schädlich H, Berg K, Emmrich F: *Age-dependent levels of select immunological mediators in sera of healthy children.* Clin Diagn Lab Immunol 1998, 5(1):28-32.

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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

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