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Predictors of Early Intervention Outcomes in Autism Spectrum Disorder

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DECLARATION

This dissertation is the result of my own work unless specifically indicated in the text as the outcome of a collaboration or cited and acknowledged as material from another source. It has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

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ABSTRACT

Early detection and intervention are theorized to facilitate better outcomes in autistic children. However, response to early intervention varies considerably between individuals, some children show significant improvement, while others show minimal response to the intervention. Some pre-treatment individualized characteristics as well as intervention-specific factors were theorized to moderate outcomes, but literature revealed often mixed findings making difficult to understand whether and to what extent these factors facilitate learning during treatment. To parse the heterogeneity of the autistic population, recent studies have also attempted to investigate biological factors related to clinical and behavioral profiles. However, to date it is unknown whether and how biological information can provide insight into the variability of treatment outcomes. With this in mind, we attempted to expand the current knowledge by first investigating whether pre-treatment child's characteristics and blood leukocyte gene expression patterns could predict developmental trajectories during treatment. Leveraging a cohort of 41 autistic toddlers who received the same early intervention and provided a blood sample, we were able to analyze the effect of starting treatment very early (i.e., <24 months) on treatment trajectories. We also provided for the first-time evidence that both pre-treatment blood leukocyte gene expression patterns and clinical-behavioral characteristics are important for predicting developmental change during intervention, and that pretreatment epigenetic mechanisms such as histone acetylation may be a key biological process that influence how a child respond to early intervention. Lastly, we carried out a mega-analysis of a large international consortium, isolating individual child characteristics and treatment related factors to examine their key role in moderating child developmental trajectories throughout the course of early intervention. The final dataset comprised 645 autistic toddlers who received two types of interventions either Early Start Denver Model (ESDM) or other treatment

as usual/community interventions (TAU/COM). This mega-analysis provided strong evidence that individual factors, such as cognitive level and age at treatment start, predict most outcomes, and on-average, ESDM may promote some developmental skills better than COM/TAU. Taken together, these results advance our understanding of "what works, for whom, for what and why" questions, identifying new biological predictors and providing an alternative methodology that can effectively examine the individual variability of treatment outcomes.

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Publications

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- Mandelli, V., Landi, I., **Busuoli**, E. M., Courchesne, E., Pierce, K., & Lombardo, M. V. (2023). Prognostic early snapshot stratification of autism based on adaptive functioning. *Nature Mental Health*, *1*(5), 327–336.

- Bertelsen, N., Landi, I., Bethlehem, R.A.I., Seidlitz, J.; **Busuoli**, E. M. … & Lombardo, M. V. (2021) Imbalanced social-communicative and restricted repetitive behavior subtypes of autism spectrum disorder exhibit different neural circuitry. *Communications Biology* **4**, 574.

- **Busuoli**, E. M., … & Lombardo, M. V. The effects of early intervention type and child individual factors on developmental trajectories in autism: An individual participant data meta-analysis. *Manuscript in preparation.*

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Nomenclature and Acronyms

ASD Autism spectrum disorder

APSS Azienda Provinciale per i Servizi Sanitaria

RCT Randomized clinical trial

COM/TAU Community settings/Treatment as usual

ESDM Early start denver model

EIBI Early intensive behavioral intervention

NDBI Naturalistic Bevelopmental Behavioral Intervention

ABA Applied Behavioural Analysis

IPD-MA Individual participant data meta-analysis

ESI Early Social Interaction

ADOS-2 Autism Diagnostic Observation Schedule–Second Edition

CARS Childhood Autism Rating Scale

STAR Strategies for Teaching Based on Autism Research

ECI early childhood interventionist

aSLP Adapted Student Learning Profile

MSEL Mullen Scales of Early Learning

ELC Early learning composite

EL Expressive language

RL Receptive language

VR Visual reception skills

FM Fine motor

VABS Vineland Adaptive Behavior Scales 2nd edition

ABC adaptive behavior composite

DQ developmental quotient scores

GMDS Griffith Mental Development Scales

MSE Cross-validated mean-squared error

ACC anterior cingulate cortex

MCC middle cingulate cortex

PC posterior cingulate cortex

dMPFC dorsal medial prefrontal cortex

vMPFC ventral medial prefrontal cortex

vPMC ventral premotor cortex

SMC somatomotor cortex

TPJ temporoparietal junction

PT planum temporale

IPL inferior parietal lobule

IPS intraparietal sulcus

pSTS posterior superior temporal sulcus

ATL anterior temporal lobe

MTG middle temporal gyrus

Ins lateral occipital cortex, and insular cortex

pSTS posterior superior temporal sulcus

Chapter 1: Introduction

1.1 Heterogeneity in Autism Spectrum Disorder

Autism spectrum disorder (ASD), henceforth 'autism', is one of the most common neurodevelopmental disorders, characterized by core challenges in social communication, as well as restricted interests and repetitive behaviors and differences in sensory function (*Diagnostic and Statistical Manual of Mental Disorders*, 2013). However, autistic individuals may also have difficulties in adaptive behavior, language and cognitive skills. Current estimates suggest that about 78 million people meet the criteria for autism worldwide (Lord et al., 2022), although this prevalence varies by biological sex, with males being more likely (approximately four times more) to be affected (Zeidan et al., 2022). While the diagnostic criteria attempt to maximize clinical consensus, they also mask a wide heterogeneity in terms of severity of symptoms, level of cognitive and communication abilities. But going beyond, the hallmark of heterogeneity in autism is its multilevel-level presentation, applicable in biology, outcomes and treatment responses, and it is not only present between autistic individuals but also within individuals across development (Charman et al., 2017; Lombardo, Lai, et al., 2019; Lombardo & Mandelli, 2022).

One of the most important reasons why understanding heterogeneity is crucial is to be able to account for the wide variability of treatment responses in autistic individuals, whereas some children make significant gains after intervention, while others show a minimal or no response (Godel et al., 2022; Lombardo, Lai, et al., 2019). Considerable attention has been given to early

behavioral intervention, since most of the clinicians, researchers and also autism community agree that behavioral interventions delivered during early periods should be available to help autistic children grow and realize their potential and to increase the likelihood of long-term life satisfaction (Leadbitter et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Although literature has presented mixed and heterogenous findings, early interventions are considered evidence-based treatment approaches developed for autistic children. Some reviews and meta-analyses showed significant improvements on cognition, adaptive abilities and language after intervention (Fuller et al., 2020; Rodgers et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020; Warren et al., 2011), while others displayed limited treatment effects (Crank et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Given the significant amount of time and resources required for the intervention, enrolling autistic children in intervention programs from which they will not benefit leads to negative implications for the children, their families, and the community. However, the limited knowledge of variables that influence treatment response makes very difficult to predict to what extent a child will improve during treatment. Until now it is unclear whether and to what extent factors such as the type of intervention approach, treatment specific components and child's characteristics facilitate learning during treatment. (Godel et al., 2022; Vivanti, Prior, et al., 2014). The lack of one single treatment approach which works for all autistic children and the wide individual variability in treatment response suggest to develop more individualized guidelines for intervention, which allow to choose the appropriate program based on the individual profile of the child at the beginning of treatment (Vivanti et al., 2013; Vivanti, Prior, et al., 2014). In this regard, recent works have also focused on identifying biological factors able to parse the heterogeneity of the autistic population in order to provide information on the variability of treatment responses and create more personalized guidelines (Lombardo, Eyler, et al., 2019, 2021; McPartland et al., 2020). But to date, no

valid biological factors have been found to be significantly related to clinical and behavioral profiles which are meaningful for clinical practice (Lord et al., 2020, 2022). In line with the precision medicine framework, the present work aims to extend and improve upon previous findings to address not only the question of treatment effectiveness at the group level, but also to focus on individual responses and how to predict them before the intervention starts.

Early intervention approaches are considered effective when they help to maximize the child's potential by facilitating the development and learning new skills important for supporting independence before the child reaches school age (Rodgers et al., 2021). The treatment outcome measures used among studies are usually related to specific intervention targets or scores on broader standardized assessments of developmental skills. However, most previous researches in this area has often focused on distal measures that capture specific developmental domains and core autism features, such as changes in standardized tests of autism symptom severity, adaptive, cognitive and language skills, which are usually considered important for demonstrating the effectiveness of intervention (Chen et al., 2022; M.-C. Lai et al., 2018). Although the constructs examined as short-term outcomes are consistent across studies, there are significant differences in the choice of which predictors are reported. And even when similar sets of predictors and outcomes are investigated, the role of predictors is highly variable. An important source of inconsistency present among studies is due to different methodological and measurement choices. Previous studies have reported that how researchers operationalize and measure intervention goals in social communication affects whether intervention effects are found or not (Colombi et al., 2019; Yoder et al., 2013). Also for measuring cognitive functioning, for example, there are multiple instruments used, and there is considerable inconsistency in the way scores are used for statistical analysis (Towle et al., 2020). Therefore, the use of different outcome measures and the choice of different predictor variables among studies have a large impact on the variability

of findings (D. Zachor & Ben-Itzchak, 2017). Here the focus is on the most studied pre-intervention factors, available in the common practice, for predicting each of the outcome domains commonly used in autism intervention research, including autism severity, cognitive abilities, and adaptive behavioral abilities. To reduce to some extent the additional factors contributing to the heterogeneity of treatment outcomes, the findings listed below come from the most widely adopted evidence-based intervention approaches, that will be addressed also in the next chapters, such as Early Intensive Behavioural Intervention (EIBI) and related variants (Lovaas, 1987a), and the Naturalistic Developmental Behavioural Interventions (NDBIs) (Schreibman et al., 2015).

This introductory chapter provides a brief critical overview of predictive factors of early intervention outcomes to better elucidate the starting point for improving the knowledge on *"what works, for whom, for what and why"* questions (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Vivanti, Prior, et al., 2014). Specifically, the extent to which factors such as clinical and biological characteristics, and specific treatment components may influence outcomes in the context of early intervention is examined. These predictors might be associated with outcomes in two ways: prognostically when factors hold some value in predicting child developmental change over time regardless of the type of intervention received; or predictively when the influence of certain characteristics on treatment response varies according to the type of intervention program received (Bent et al., 2023; Vivanti, Prior, et al., 2014). To enable this distinction, research on predictors of treatment outcomes should use study designs that compare different intervention programs to distinguish between moderators of response, specifically related to the treatment type received, and general prognostic factors, which are associated with outcomes (Vivanti, Prior, et al., 2014). Although this approach is important from the perspective of precision medicine, only a few studies were able to report the specificity of predictors to one treatment program versus another (Bent et al., 2023; Rodgers et al., 2020a;

Rogers et al., 2019, 2021; Stahmer et al., 2011). Most previous studies examining moderating factors of treatment outcome have discrepancies in reporting practices, a narrow set of individual participant characteristics, and a limited statistical power. All these limitations may have hidden all the existing associations between participant-intervention factors and intervention effect (Crank et al., 2021; Lord et al., 2022), thus is still very difficult to determine whether a factor that moderates child development during treatment is sensitive only to the context of that specific treatment.

1.2 Treatment specific factors

Treatment response is expected to be associated with treatment-related factors, but it is not yet entirely clear how these factors work. Although numerous studies have been conducted on early intensive interventions, findings still differ widely, making it hard to identify which treatment components promote a positive change in the child (Rodgers et al., 2020a). Currently, it is difficult to understand which intervention approaches, intensity, and duration foster the greatest effects, since in all types of evidence-based early intervention, the inter-individual variability of treatment response is high.

1.2.1 Treatment type

To date, there is no pharmacological treatment for the main symptoms of autism, and the nature of interventions intended to help autistic children is mainly psychoeducational, psychosocial, or behavioral (Landa, 2018). Among these types of interventions, there is no standard treatment for autism that is unanimously accepted as the one that works best. The most frequently used programs are

"treatment as usual" (TAU), which are community-based treatments that follow local guidelines. They usually include a combination of interventions based on different non-autism-specific special education services, such as occupational therapy, sensory integration therapy, speech therapy and classroom aide. However, these approaches have limited effectiveness and are not evidence-based, thus more structured and comprehensive interventions are usually preferred (Asta & Persico, 2022). The primary most studied evidence-based approaches to early intervention are: EIBI and NDBI. This work solely focuses on results from these two approaches NDBI and EIBI and related variants, findings from approaches other than those mentioned above are not included, as they are beyond the scope of this work. EIBI is a package of manualized treatment, delivered on an individual basis for 20-40 hours per week, using techniques and technologies guided by the traditional principles of applied behavior analysis (ABA) (Reichow et al., 2018). The set of techniques used (such as breaking skills down into their basic components) focus on stimulus discrimination and positive reinforcement in order to promote learning and shifting the child's developmental trajectory toward a more positive one during the early period (Lovaas, 1987a; Rodgers et al., 2020b). Treatments based on ABA principles have become progressively more popular, especially after the publication of the promising results of Lovaas' study in 1987 (Lovaas, 1987a) where the use of EIBI, with a specific teaching procedure such as discrete trial teaching (DTT), promoted higher cognitive and educational functioning than children receiving less intensive behavioral intervention or other types of treatment. Since then, studies using ABA principles have multiplied (Asta & Persico, 2022). To the point that, this intervention approach has become the most studied for the autistic population in the past 40 years, and therefore, EIBI and other highly structured ABA-based interventions are more commonly recommended for children with autism (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Several meta-analyses have been conducted, most of them found that children who received EIBI treatment had better outcomes than children in the comparison groups; the most pronounced improvements were

observed mainly in adaptive behavior and cognitive functioning (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020) and language (Makrygianni & Reed, 2010; Reichow et al., 2018). Taken together, highly structured ABA-based interventions studies supported their effectiveness in improving a wide range of outcomes, however only a small fraction of past studies have explored the treatment effects using randomized clinical trials (RCTs) designs and masked personnel and participants; moreover the majority of outcomes were taken from caregiver report (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Thus, the current state of evidence on EIBI and related ABA-based intervention variants, suggests that robust conclusions on the effectiveness are still limited by the low methodological quality of primary studies (Reichow et al., 2018).

Meanwhile, the current field is expanding the knowledge by including NDBI approaches, which are considered a subsequent adaptation of the original EIBI model that incorporates traditional ABA-based principles within a more naturalistic and developmentally informed framework (Rodgers et al., 2021). These approaches emphasize the use of strengths-based models to teach skills in line with developmental principles, within a social context that is emotionally meaningful to the child. For example, using natural rewards that incorporate the child's interests and choices during play or other daily activities (e.g., when the child says "car", he is rewarded by giving him a toy car to play rather than a candy for the correct labelling) (*2021-2023 IACC Strategic Plan for Autism Research*, 2023; Schreibman et al., 2015). Examples of NDBI models which have been most studied include Early Start Denver Model (ESDM), pivotal response training, Project ImPACT (Improving Parents As Communication Teachers), Joint Attention Symbolic Play Engagement and Regulation (JASPER), enhanced milieu teaching, Incidental Teaching, reciprocal imitation training, and Early Social Interaction (ESI) model. Although these naturalistic intervention packages share common theoretical underpinnings, they differ from each other since some

are more comprehensive interventions, addressing a wide number of developmental domains (e.g., ESDM), while others are more targeted interventions, addressing a specific developmental area such as social communication (e.g., JASPER) (Schreibman et al., 2015). In previous meta-analyses this emerging category of interventions has shown promising effects across a wide range of developmental domains such as social communication, language and cognitive skills (Crank et al., 2021; Fuller et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Tiede & Walton, 2019); moreover when compared with other intervention approaches it has shown to be the most supported approach by RCT studies providing evidence of improvement in social communication and language skills. NDBI studies are also the least prone to use caregiver report as the primary measure of intervention effectiveness; indeed, excluding low quality outcomes which come from caregiver reports, the development of social-communication skills remains significant (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Compared with other intervention approaches for autistic children, this body of evidence is relatively methodologically rigorous. However NDBI studies are still constrained by some methodological and quality limitations, such as the prevalence of outcomes subject to high detection bias and the use of proximal and context-bound outcome measures (Crank et al., 2021; Fuller et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Thus, further studies are needed to accumulate evidence of NDBI effects.

In the theoretical framework of NDBI approaches, the ESDM (Rogers, S. J., & Dawson, G., 2010) has emerged as a promising and cost-effective early intervention for the needs of autistic children as young as 12 months. (Cidav et al., 2017). Using a set of manualized treatment procedures and a comprehensive curriculum, ESDM aims to facilitate improvement in multiple developmental domains and in key areas involved in autism challenges (Fuller et al., 2020; Rogers, S. J., & Dawson, G., 2010, p. 201). The intervention program can be

delivered either individually or in groups (Vivanti, Paynter, et al., 2014) by trained professionals or parents (Rogers et al., 2012) in-person or via telehealth (Vismara et al., 2018); and the level of intensity can be relatively low or high (Cidav et al., 2017; Colombi et al., 2018; Estes et al., 2015; Rogers et al., 2021; Wang et al., 2022). Research on the ESDM includes several RCT studies, some have reported that participation in the ESDM intervention resulted in significant improvements, compared to control groups, in cognitive functioning (Dawson et al., 2010, 2012; Estes et al., 2015), autistic symptoms (Dawson et al., 2012; Estes et al., 2015), adaptive behavior (Dawson et al., 2010, 2012; Estes et al., 2015), and language abilities (Dawson et al., 2010, 2012; Rogers et al., 2019). However, not all RCT studies reported similar results, some showed partially replicated or nonsignificant results in similar treatment outcomes (Rogers et al., 2012, 2019, 2021).

Systematic reviews of the empirical research on the ESDM have also shown overall positive results. Waddington and collaborators included 15 studies using a range of group and single-case designs, and reported promising findings in cognitive skills and language abilities, while results for adaptive behaviors and autism symptom severity were mixed (Waddington et al., 2016). A second review of 10 studies found that despite the wide variability of study designs, intervention agents, setting, intensity and duration, children receiving ESDM intervention showed improvements. Significant gains were found in the areas of cognitive functioning, language, adaptive behaviors, and social communication, however findings regarding whether the ESDM was more effective than other treatment options were mixed (Baril & Humphreys, 2017). Notably most of studies included in these works were considered limited in terms of quality and rigor. To expand and refine results of these reviews a number of meta-analyses were conducted by including more recently published studies, focusing on comparative studies; and using a meta-analytic approach that allowed quantitative understanding of effects on specific outcome domains. (Fuller et al., 2020; Wang et al., 2022; Yu et al.,

2020). Fuller and colleagues (2020) conducted a systematic review and meta-analysis of the effects of ESDM intervention including 12 studies (quasi experimental or randomized control trials). Findings revealed that participants who received the ESDM showed significant more gains in cognition and language domains compared to children in the control group, however no significant effects for autism symptomology and adaptive behavior were observed. Despite the overall positive effects reported in language and cognitive areas, this result should be taken with caution, because of the high heterogeneity observed in the sample and the limited scientific rigor of the study designs (Fuller et al., 2020). Another meta-analytic work based on 14 RCTs aimed to investigate differences when comparing types of ABA-based interventions such as applied behavior analysis (ABA), ESDM, discrete trial training (DTT) and Picture Exchange Communication Systems (PECS). Authors found that the small number of studies included in the analysis limited the ability of examining each type of intervention's strengths and weaknesses in terms of developmental outcomes (i.e. only five ESDM studies were included in the comparison with other interventions) (Yu et al., 2020). Therefore, they could not provide a reasonable conclusion on the superiority of the ESDM compared to other mainstream ABA-based approaches, which is in line with the current available literature (Bent et al., 2023; Rogers et al., 2021, p. 202; Vivanti & Stahmer, 2021). Recently Wang and collaborators (2022) evaluated the effect of ESDM in Western and Asian countries, including 11 high-quality RCTs. Findings suggested that ESDM intervention led to significant improvements in cognitive domains, autism symptoms, and language (Wang et al., 2022). Results for cognition and language are consistent with previous meta-analyses (Fuller et al., 2020), suggesting that ESDM promotes more positive progress in these domains, while the improvement in autism symptoms is not consistent with previous results reported by Fuller and colleagues. This difference might be related to the fact that Wang and collaborators employed only RCT studies, while previously quasi-experimental designs were also included (Fuller et al., 2020). Furthermore, the use in Asian

countries of the Childhood Autism Rating Scale (CARS) to measure autism symptoms instead of the Autism Diagnostic Observation Schedule-Second Edition (ADOS-2) (Lord et al., 2000) may have caused an increase in effect size, since the CARS measurement is a rating scale that is a more subjective tool. To conclude, authors tried to explore potential moderating variables of intervention effects, they found that the effect sizes of autism symptoms and language were moderated by country (Asian countries had larger effect sizes than in Western countries), but they failed to find any other relevant relationship between moderator variables due to the low number of studies (Wang et al., 2022).

To improve methodological quality of analyses, it seems necessary to consider more data from different studies, and to include as few measurement tools as possible, to ensure comparability among participants (Eckes et al., 2023; Wang et al., 2022). Although RCT studies are the gold standard for meta-analysis, providing the least-biased estimates of efficacy, results should be interpreted cautiously due to the small number of studies present in the literature and the possible low statistical power resulting from them. Past literature has shown that small sample size means that the variability in estimated effect size is consistent, irrespective of what the true effect is (Lombardo, Lai, et al., 2019). Therefore, the picture depicted by RCTs, if not including a large number of studies, may not be representative of the autistic population. Furthermore, to assess more precisely the treatment effect, the investigation of multiple potential moderating variables is required, because the variability of individual responses within the same type of intervention is high and, as described above, often varies across studies leading to different conclusions about group-level efficacy/effectiveness (Rogers et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020). To better understand the intervention response, it is necessary to know whether the difference between treatment groups varies based on pre-treatment characteristics, i.e., allowing analysis of the statistical interaction between selected characteristics

and treatment type (Lord et al., 2005). However, analyses which attempt to identify child and intervention factors as potential moderators of treatment outcomes are scarce and inconclusive. This is due to the inconsistent reporting of informative predictors, the insufficient sample sizes and also because RCTs often intentionally set strict inclusion and exclusion criteria to control for variability (i.e. children with a very low cognitive skills are often excluded from studies) (Colombi et al., 2019; Lord et al., 2022; Russell et al., 2019). Overall, low quality of evidence seems to be a common problem in autism intervention studies, and this limits the informative value of studies in this field, suggesting that to date there is limited evidence to indicate whether specific child characteristics might distinguish response to one early autism intervention approach versus another. Future research should be improved by using novel research designs to answer the question '*What works for whom?*' (Bent et al., 2023; Crank et al., 2021; Eckes et al., 2023; Rodgers et al., 2021).

1.2.2 Treatment intensity

Many professionals suggest that the delivery of intervention should be intensive (e.g. provided for 20-40 hours per week for over a year or longer) to increase the potential for improving outcomes for autistic children (Lord, 2001; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). However, the evidence supporting this widespread recommendation is often contradictory and difficult to be interpreted. These guidelines are originated from some studies that have found interventions must have a combination of early and intensive components to be effective. For example, initial study led by Lovaas and colleagues (1987a) found that children younger than 4 years old who received 40 hours per week of behavioral intervention had greater cognitive outcomes than those who received only 10 hours per week (Lovaas, 1987a). Although this was one of the first influential studies, the quality of results was affected because subjects were not randomly assigned to groups. Subsequent studies have further

investigated the importance of more intensive intervention during the critical period of development. Granpeesheh and collaborators (2009) have assessed treatment progress for 245 autistic children receiving EIBI services; results showed that children aged 2 - 7 years made significant gains in the acquisition of new skills as treatment hours increased, while children in the older group 7-12 years no significant relationship was found between treatment hours and the number of behavioral goals mastered (Granpeesheh et al., 2009). Consistently with these results, a randomized controlled trial have reported that more hours of intervention for both ESDM and TAU groups predicted significantly better scores on cognitive outcomes and autism severity scores, for toddlers at risk for autism aged 14 to 24 months (Rogers et al., 2012). Therefore, the combination of early age and high intensity together seems to significantly influence treatment outcomes. However, evidence on how much and for how long a given early intervention should be delivered is scant, and only a few systematic comparisons addressing these questions have been conducted to date (Lord et al., 2022). With this aim, Rogers and colleagues conducted a recent study (2021) including three different sites with 2 years old autistic children. They compared two types of intervention at two different moderately high intensities (15 or 25 hours of ESDM; 15 or 25 hours of EIBI) for 12 months. Findings revealed that overall treatment type and intensity have no main effects on child outcomes, but there was a little evidence that initial severity moderated effects of treatment intensity on autism severity change, in one of the three sites included in the analysis (Rogers et al., 2021). On the other hand, another methodologically rigorous early intervention study has tested whether lower intensities can promote significant outcomes. Pickles and collaborators have tested the long-term follow-ups of Preschool Autism Communication Trial (PACT) (J. Green et al., 2010) a parent-mediated social communication intervention for children aged 2–4 years delivered at low intensity. Findings revealed that even at lower intensities early intervention, such as PACT, can promote improvements in core autism symptoms (Pickles et al., 2016).

Notably, several meta-analysis studies have been carried out to investigate within large scale studies, whether the duration and intensity of the intervention moderate treatment effects. Overall, the results reported so far are widely heterogeneous. For example, some initial meta-analysis showed that treatment intensity was associated with treatment gains in both cognitive functioning and adaptive behavior outcomes (Eldevik et al., 2010; Makrygianni & Reed, 2010). A recent meta-analytic work (2020a), with comparative studies including both RCTs and non-randomized comparative studies, found that receiving early high-intensity ABA-based intervention leads to slightly greater improvements in cognitive and adaptive behavior scores than those receiving low-intensity, TAU or eclectic interventions (Rodgers et al., 2020a). Similarly, another meta-analysis of ABA-based interventions, with mostly quasi-experimental designs, showed that the influence of treatment intensity on adaptive behavior outcome is significant but it decreases with older age (Eckes et al., 2023). However, the validity of all the examinations described is limited by the fact that quasi-experimental studies included in meta-analyses cannot provide reliable results about the causal effect of intervention factors.

With this aim, a meta-analysis of early interventions, which reviewed only RCTs and excluded studies with a high risk of bias, found that the intensity of early intervention and duration did not reveal any significant effects. However, this could be ascribed to the fact that the unequal number of studies and participants included in the subgroup analyses may have limited the power to identify any actual effects of this moderator on treatment outcomes (Daniolou et al., 2022). The lack of evidence that treatment intensity moderated outcomes is also reported by NDBI meta-analyses, which have investigated the effect of cumulative intensity on cognitive, adaptive behavior, autism symptomatology and language outcomes (Crank et al., 2021; Hampton & Kaiser, 2016; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020). More specifically,

Fuller and collaborators have examined the effect of intervention-level characteristics in ESDM studies on treatment responses. Also here, authors found that neither the length nor intensity of the dosage were significantly related to the magnitude of the outcomes (Fuller et al., 2020). As suggested by the authors of aforementioned meta-analyses (Crank et al., 2021; Fuller et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020), it should be noted, that the inability to find evidence supporting this association is not definitive proof of its absence. The association between treatment intensity and gains in autistic children is indeed likely complicated by other factors, such as the risk of small study bias, the risk of misreporting total intervention hours in parent-implemented interventions studies and the study quality weakness due to quasi-experimental non-randomized study designs. All of these factors may have limited the ability to detect a potential association between intervention intensity and outcomes (Crank et al., 2021). Given that, future analyses of early interventions should continue to quantify intervention components and analyze the differences they might have on outcomes.

To date, we do not have reliable high-quality data from past studies on which to base decisions about what specific type of intervention and at what intensity, treatment may have a significant impact on a child's progress. A few studies investigated whether the effect of treatment intensity varies according to treatment type (Rodgers et al., 2020a) and baseline child characteristics (Eckes et al., 2023; Rogers et al., 2021). Future work is needed to investigate the effects of treatment type and intensity according to individual child characteristics to guide clinical practice in choosing the early intervention program (Rogers et al., 2021; Vivanti, Prior, et al., 2014). This, would be critical especially when intensive treatments are too expensive, and common recommended interventions are unlikely to produce significant changes (M.-C. Lai et al., 2018).

1.3 Biological factor

Although there is a growing body of evidence showing that some treatment approaches provide support for autistic children, it remains unknown how and why evidence-based treatments work, both in terms of mechanisms of change and active ingredients (Lord et al., 2022). Although the precision medicine approach in autism intervention may not rely on biomarkers, the question of which underlying biological features may be relevant for predicting treatment outcomes remains. To test this question, recent works (Lombardo, Eyler, et al., 2019, 2021; Loth et al., 2016, 2017; McPartland et al., 2020) have focused on identifying biological factors able to stratify the autistic population, which could also provide information on the variability of intervention responses. This area of research aims to use biomarkers (e.g., neurobiological factors) as key targets to inform behavioral interventions and create more personalized guidelines. However, such targeted treatments for specific biological subtypes in autism have not yet been developed, as to date no valid biomarkers have been found to be significantly predictive of clinical and behavioral features to be translated into routine clinical practice (Lord et al., 2020, 2022).

Furthermore, an important goal for the current research field is to show whether and how early intervention influences the brain development. Previous studies have described how the development of brain circuits for social and language skills is shaped by the interaction between the child and the social environment during early sensitive periods (Dawson, 2008; Kuhl, 2007; Kuhl et al., 2005; Mundy & Rebecca Neal, 2000). Therefore, investigating which biological mechanisms are responsible for behavioral changes during environmental manipulation, such as early intervention, it may reveal a better understanding of why some individuals improve more than others during the intervention. With this regard, although the literature is scarce, one promising way

to provide a comprehensive understanding of the mechanisms behind the effectiveness of intervention, is to integrate biological measures into the design of treatment studies (Cicchetti & Curtis, 2015; Dawson et al., 2012).

Neuroimaging and neurocognitive biomarkers studies, including magnetic resonance imaging (MRI), functional near-infrared spectroscopy (fNIRS), electroencephalography (EEG) and eye tracking are growing in the field of autism research (Jones et al., 2019; Webb et al., 2020, p. 201). These tools have also been embedded into early intervention RCTs to elucidate the underlying neural mechanisms that may predict changes during development (McGlade et al., 2023).

For example, Dawson and colleagues (2012) have conducted an RCT study, where they showed the efficacy of ESDM for autistic toddlers and they have also included EEG activity measures as treatment outcomes (Dawson et al., 2012). This study has demonstrated for the first time that early intervention, such as ESDM, is related with normalized brain activity patterns, which are correlated with gains in social behavior. However, this study has not included pre-intervention measures, thus is not possible to examine the EEG changes related to intervention, and it is unknow whether brain activity patterns moderate the response to treatment. The first systematic review that examined whether early interventions lead to changes in neurocognitive markers of EEG and eye tracking (McGlade et al., 2023), concludes that the findings were inconsistent due to small numbers of participants and methodological concerns.

Since autism represents a highly heterogeneous condition that differs in cause, course, response to treatment, and outcome (Lombardo & Mandelli, 2022); further studies are needed to understand the biological processes related to developmental changes. This would help to create and choose more tailored intervention approaches, designed for individuals that have similar biological characteristics (Dawson, 2008). Although this goal is still in development, it

represents a shift toward precision medicine based on individual biological factors, which it is hoped will ultimately help untangle the complexity of heterogeneity in treatment responses.

1.4 Pre-treatment clinical-behavioral and demographic characteristics

There is an extensive literature investigating pre-treatment clinical-behavioral and demographic characteristics to answer the questions about *for whom* and *when* early interventions are most effective. Most of these studies do not inform about treatment-specific response, but rather indicate the relationship between child characteristics and developmental changes during treatment (Lord et al., 2005). Although previous studies have found various individual factors to be potentially predictive of outcomes, there is still no clear consensus on what the active elements of change are in the context of early intervention (Vivanti, Prior, et al., 2014). Therefore, the question of which individual characteristics are associated with children's intervention outcomes often remains unanswered, as available information diverges among studies.

1.4.1 Age at treatment start

The age at which a child starts intervention has been often identified as a prognostic factor for several treatment related outcomes. Scholars and practitioners have consistently stated that intervention should be delivered as early as possible, starting at the diagnosis of autism or even earlier, because the earlier children start treatment programs, the better the outcomes (Reichow, 2012; Towle et al., 2020; Warren et al., 2011). For this reason, early diagnosis is now strongly

encouraged since early behavioral signs begin to manifest before 18 months (Pierce et al., 2011, 2021) and autistic children can be diagnosed by 24 months (Zwaigenbaum et al., 2015). However, the possible advantages of early diagnosis and treatment are the focus of an ongoing debate because the evidence supporting "earlier is better" are mixed and sometimes difficult to interpret due to the complex relationships between all the predictor variables involved in the outcomes. For example, the cognitive functioning at baseline is an individual factor commonly associated with later outcomes, so it may overlap with the individual effect of early age start, if the shared variance between these two variables is not controlled with statistical or design methods (Siu et al., 2016; Towle et al., 2020). Uncertainties also include community-based or "treatment as usual" (TAU) interventions, which are usually associated with significantly weaker gains than highly controlled intervention studies in academic settings. For this reason, it remains unclear whether children undergoing community-based intervention benefit from earlier diagnosis and intervention. Generally, the replicability of effectiveness of early intervention in nonacademic community settings needs further investigations (Nahmias et al., 2019; T. Smith et al., 2015a; D. A. Zachor & Ben Itzchak, 2010).

Moreover, there are studies have reported that chronological age is not associated with most common outcomes associated with treatment, such as autism severity, adaptive, cognitive and language skills. For example, Eapen and collaborators investigated predictors of outcomes in 49 preschool children enrolled in ESDM group delivered intervention. In their findings age at onset was related to parent-reported autism symptoms measures, but they did not found any association with change scores on any other outcomes examined in the study (Eapen & Crncec, 2016; Towle et al., 2020). Vivanti and colleagues examined the effectiveness of the ESDM in a long-day care community setting in 27 autistic preschoolers compared to a different treatment program (a similar community long-day care service) received by 30 peers. They found that chronological age

did not predict cognitive, adaptive behavior and autism severity scores (Vivanti, Paynter, et al., 2014). Also, Contaldo and colleagues (2020) provided individualized and group ESDM sessions to 32 autistic children and found no significant associations between age of the child at intake and language skills developed during treatment (Contaldo et al., 2020). Hayward and collaborators (2009) examined outcomes after one year of intensive one-to-one University of California at Los Angeles Applied Behavior Analysis (UCLA ABA) treatment, provided by therapists (n=23), or intensive treatment model, managed by parents (n=21). They found that all correlations between age at start and outcome measures such as cognitive, language and adaptive behaviour abilities were non-significant (Hayward et al., 2009). Also in a systematic review of 11 EIBI studies, authors reported that chronological age was not associated with cognitive outcomes in any of the studies assessed (Howlin et al., 2009).

With regards to meta-analysis findings, a recent work led by Crank and collaborators (2021), evaluated the extent to which NDBI intervention effects vary as a function of chronological age. They found that none of the summary effects on cognitive, adaptive behavior, language and autism symptomatology outcomes were moderated by the child's age at the start of the intervention, thus no evidence was found that developmental gains during NDBIs lessen with the progress of age, at least until the age of 6 years (the highest mean age in the included studies) (Crank et al., 2021). With regards to language outcomes (expressive language, receptive language and composite language score), previous meta-analytic work (Hampton & Kaiser, 2016; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020) found that age at baseline is not a significant moderator of intervention effect on language skills. Furthermore, in one of the largest individual participant data meta-analysis (IPD-MA), authors have evaluated the effectiveness of early intensive ABA–based interventions for 491 pre-school autistic children compared with TAU or eclectic intervention. Their findings suggest that there is a lack of conclusive evidence whether age may

influence the effectiveness of ABA-based or eclectic intervention on cognitive and adaptive behavior outcomes, however all analyses had very wide confidence intervals indicating a substantial uncertainty of findings (Rodgers et al., 2021).

By contrast, to support early intervention decision making, there are several rationales, which recommend combining both early start and intensive level of intervention, regardless of the type of treatment and the specific child symptoms or behavioral characteristics (Godel et al., 2022). First of all, early intervention is designed to take advantage of experience-dependent neuroplasticity during critical periods (Piven et al., 2017). Early neuroplasticity is a crucial brain property which creates and organizes neuronal connections and enables learning in response to the child's experiences with the environment. Through the interaction with the environment the brain is able to process synaptic connections and cortical specialization (Johnson, 2011; Nelson, 2000). Thus, early intervention can be considered as a highly specialized enrichment environment that, through experience, potentially allow for greater developmental improvements (Dawson, 2008). In addition to the early neuroplasticity theory, there are several empirical considerations that point to the relevance of the timing of intervention in various models of early interventions.

The effect of age at treatment start was also examined in a complete crossover RCT study with 82 autistic children enrolled in the Early Social Interaction (ESI), a model implemented by parents. The authors investigated the effects of individual-ESI when it begins at 18 or 27 months of age and also comparing with the effects of group-ESI, which is less intensive, as an active control condition. Children assigned to individual ESI at 18 months exhibited larger improvements than those who started individual ESI at 27 months in social communication, receptive and expressive language and daily living skills. This RCT showed that even a small-time difference of 18 months compared with 27 months, may have an impact on child outcomes. This result was not present in the

group-ESI, excluding the possibility of maturation effects (Guthrie et al., 2023). To understand timing effect of community-based early intervention, Gabbay-Dizdar and collaborators have assessed longitudinal changes in core autism spectrum disorder symptoms of 131 children, diagnosed at 1.2–5 years of age. Findings showed that the percentage of children who improved in autism symptom severity was significantly higher in the group of children diagnosed before 2.5 years (65%) compared to the children diagnosed after this age (23%) (Gabbay-Dizdar et al., 2022). In another study Smith and collaborators (2015a) examined EIBI in nonacademic community agencies including 71 autistic children. They found that even after controlling for all other covariates (autism severity, cognitive and adaptive skills), lower age at baseline predicted better outcomes for cognitive, adaptive behavior skills and autism symptom severity scores (T. Smith et al., 2015a). Also, in an RCT study of parent-delivered implementation of ESDM, in 98 toddlers at risk for autism, authors found that for both ESDM and TAU groups younger age at intake predicted higher cognitive outcomes (Rogers et al., 2012). Another RCT compared ESDM in an inclusive classroom setting (n= 22) versus specialized classrooms (n= 22). The role of age at treatment start in predicting various outcome measures was analyzed for each group. Across both inclusive and specialized settings age of starting was significantly associated with verbal cognition, where younger children have higher gains in language skills than older children. No significant associations were found between the other outcome measures (adaptive behavior, non-verbal cognitive outcomes) and age at intake. (Vivanti et al., 2019). The same group, led by Vivanti and colleagues, tested the hypothesis that preschoolers who fall within the age range suggested by ESDM (i.e., 18-48 months) have more favorable outcomes than children involved in the same ESDM program but older than 48 months. They included 32 children aged 18–48 months and 28 children aged 48–62 months receiving the ESDM for one year. Findings suggested that both groups undergoing significant improvement with respect to non-verbal cognition, autism symptom severity and adaptive behavior skills. However, the younger group of

children get higher verbal skills compared to the older group, and the association between verbal cognition score and age at start was moderated by verbal skills at the baseline (Vivanti et al., 2016).

To conclude, because of the vast number of studies and meta-analyses with conflicting results, it remains to be clarified whether children who start treatment at a younger age have greater gains than those who receive treatment later in life; and if advancing age limits the amount of change that can be achieved with the intervention during childhood. However, early diagnosis and early intervention continue to be of paramount importance, but further research will be needed to clarify the equivocal nature of the existing literature.

1.4.2 Cognitive level at the baseline

Children's cognitive level is considered one of the most reliable prognostic factors of later outcomes. Individual differences in cognitive abilities are correlated with important life outcomes, including educational and occupational achievement and health (Davies et al., 2011). Available evidence indicates that pre-treatment cognitive skills are also the most often reported predictors of outcomes in the context of autism early intervention (Hayward et al., 2009; D. Zachor & Ben-Itzchak, 2017). Cognitive ability is considered a highly heritable trait, although it can be influenced by the learning environment, such as early intervention (Ben-Itzchak et al., 2014; Gräff & Tsai, 2013). For this reason, cognitive domain is often examined both as a predictor and as an outcome measure.

Usually the higher initial cognitive level is believed to have the greatest influence on outcome domains, creating a "rich getting richer" phenomenon, in which children with significant developmental delays show less treatment benefit, than their more advanced peers (Sandbank, Bottema-Beutel, Crowley, Cassidy,

Feldman, et al., 2020; Towle et al., 2020). One of the first study showing favorable long-term outcomes of EIBI conducted by Lovaas and collaborators, showed that children with more positive outcomes in terms of intellectual and educational functioning had a higher cognitive level at treatment start (Lovaas, 1987a). Since then, studies on cognitive predictors have increased in number, but they were not always consistent in their results.

For example, a large study of 332 children, aged 2–7 years, enrolled in the community-based intensive behavioral intervention, has explored the degree to which child's characteristics at intake are related to children's outcomes. Authors found that initial cognitive level was the strongest predictor of cognitive, adaptive behaviour and autism severity outcomes (Perry et al., 2011). Howlin and collaborators (2009), in a systematic review of EIBI have found that cognitive ability at baseline was related to progress in 4 out of 11 studies included (Howlin et al., 2009) and similarly, in a more recent systematic review, cognitive skills appeared to be predictive of greater gains in EIBI approaches (Asta & Persico, 2022). Furthermore, Ben-Itzchak, Watson and Zachor (2017) compared the influence of baseline cognitive skills on outcome trajectories. Children were enrolled in early ABA-based intervention and divided in higher (DQ ≥70) and lower (DQ <70) cognitive groups. Authors showed that both groups decreased in autism symptom severity, suggesting that gains in this domain are not related to the baseline cognition. However, only the high cognitive (DQ ≥70) group significantly improved in adaptive behavior abilities, while the increase in verbal skills and fine motor abilities occurred just in the lower (DQ <70) cognitive group (Ben-Itzchak et al., 2014). Remarkably, in a subsequent review on ABA-based intervention studies, Zachor and Ben-Itzchak found that cognitive skills at intake predict both adaptive behavior and autism severity outcomes (D. Zachor & Ben-Itzchak, 2017). A recent RCT tested effects of ESDM compared to TAU on 118 autistic children, investigating also if treatment effect of two groups were moderated by baseline child's characteristics. Authors found that cognitive level

at baseline moderated the effect of ESDM compared to TAU on autism severity outcome. Suggesting that as cognitive level at baseline increased, the ESDM group decreased autism severity scores compared to the TAU group, and the opposite happened as cognitive score at baseline decreased (Rogers et al., 2019). This is one of few studies that compared the effect of initial cognitive level across different treatment groups.

Unlike what has been observed in previous studies, a retrospective analysis of low intensity ESDM intervention showed that children who benefited most from early intervention were those with a lower cognitive level at baseline. Authors divided 21 toddlers at risk for autism in two groups based on cognitive score at baseline (<75) and (≥75). Findings revealed that only the group of children with cognitive scores (<75) had a significant increase in the cognitive and language scores, while in the second group with cognitive scores (≥75) did not show significant improvement (Devescovi et al., 2016). Consistently, other works focusing on ESDM intervention found an association between lower cognitive level at baseline and higher cognitive improvements. Robain and colleagues (2020), investigated the role of cognitive level at start as a predictor of treatment outcomes in 22 children enrolled in ESDM and 38 children included in community intervention. Findings showed that in both groups lower cognitive abilities at baseline were associated with higher cognitive gains over time, compared with children who had higher cognitive abilities at start. To be noted, children showing more cognitive gains over time were the ones who decrease maladaptive behaviors during treatment (Robain et al., 2020). The same group in a subsequent study investigated developmental trajectories of autistic preschoolers, who received 2 years of individualized and intensive ESDM intervention. Using a cluster analysis, authors were able to discriminate 3 groups based on their cognitive level at baseline and the rates of cognitive change over time. One group of children at baseline had higher cognitive scores and by the end

of intervention displayed greater cognitive gains, with no cognitive delay. The other two groups both had severe cognitive delay at baseline, but they had very different outcomes. The first group showed significant adaptive and cognitive skill gains (named optimal responders) while the second group, showed lower progress in cognition and adaptive behavior (named minimal responders). Notably, optimal responders already exhibited more progress in cognition and adaptive functioning than minimal responders after 6 months of intervention (Godel et al., 2022). A potential explanation for this phenomenon might be that children with lower cognitive functioning at baseline were also those with higher potential for progress, compared to children with higher levels of cognitive functioning at baseline (Robain et al., 2020). In addition, children in the lower cognitive level group may have experienced behaviors that have a strong impact on test performance at baseline, which could potentially mask the actual cognitive functioning. With improvements in other areas, such as attention and cooperation, these children may have less difficulties in presenting more fully their true cognitive potential (Ben-Itzchak et al., 2014). However, one consideration to point out is that most of the studies included here refer to short-term outcomes (6 months to 2 years at most). Therefore, it cannot be inferred that the greater magnitude of improvements evident in children with cognitive delay necessarily reflects long-term outcomes. Further long-term investigations might be useful to explain whether these changes may represent a real change in developmental trajectories.

However, not all studies agree with this conclusion, there are studies and meta-analyses which did not found any kind of associations between pre-treatment cognitive level and intervention outcomes. This is the case of some meta-analyses of ABA interventions (Makrygianni & Reed, 2010; Reichow, 2012; Reichow & Wolery, 2009; Rodgers et al., 2020a, 2021; Virués-Ortega, 2010), where authors found inconclusive evidence that baseline cognitive ability influence the developmental progress during intervention. Also, among ESDM

studies the predictive influence of baseline cognitive ability on treatment outcomes is also unclear. As mentioned above, some studies reported significant associations (Devescovi et al., 2016; Godel et al., 2022; Robain et al., 2020; Rogers et al., 2019), while others found that baseline cognitive score is not associated with treatment related gains (Rogers et al., 2021; Vivanti et al., 2013). Due to the inconsistency of meta-analyses and individual study findings, a definitive statement on the prognostic value of pre-treatment cognitive level is needed.

1.4.3 Biological sex

Previous literature indicates the existence of a number of sex-related differences in the diagnosis and presentation of behavioral and clinical characteristics of autism (Crank et al., 2021). Prevalence data have estimated that males are 3-4 times more likely to receive an autism diagnosis than females (Loomes et al., 2017); and girls are often diagnosed at a later age than boys (Begeer et al., 2013). One possible explanation of prevalence and age at diagnosis differences may be the different presentation of core symptoms and developmental outcomes in boys and girls (M.-C. Lai et al., 2015; M.-C. Lai & Szatmari, 2020). For example, autistic girls may show a different type of restricted interests and repetitive behaviors than boys (M.-C. Lai et al., 2015), a recent work suggests that items that best discriminate boys are the stereotyped behaviors and restricted interests, while items which best discriminate girls are those related to compulsive, sameness, restricted, and self-injurious behaviors (Antezana et al., 2019). In terms of social communication domain differences, it has been reported that from early age autistic girls tend to imitate, interact and engage more in imaginative and pretend play than autistic boys. These different behaviors may mask other difficulties that females might have in social

communication abilities (R. M. Green et al., 2019). Furthermore, females are reported to "camouflage" autism symptoms more than males, it is shown that autistic girls with higher cognitive levels are those often underdiagnosed while girls who exhibit behavioral problems or low cognitive ability are more likely to get a diagnosis (Bargiela et al., 2016; Pathak et al., 2019). In an observational-clinical assessment setting such as the ADOS (Lord et al., 2000) camouflaging may lead to less severe scores, since social-communication atypicality is hidden from the assessor (M. C. Lai et al., 2019; Waizbard-Bartov et al., 2021). In addition to these reasons, it should be noted that diagnoses are based on definitions and diagnostic tools developed and validated using predominantly male samples, so the real ratio of males to females in autism is presumably underestimated.

Since biological sex accounts for important heterogeneity in autism (M.-C. Lai et al., 2015, 2018), it seems reasonable to investigate whether treatment outcomes may also vary between males and females. With this aim, some intervention trials have tested if there is a significant relationship between sex and treatment outcomes, however they did not find a clear association between these two variables (Crank et al., 2021; McVey et al., 2017). These results should not be considered definitive because of the very little exploration of this topic and the small samples of females in the existing trial literature, which make the power to examine the moderating effect of sex insufficient (M.-C. Lai et al., 2018; McVey et al., 2017). More evidences are needed to develop evidence-based sex-informed intervention for autistic individuals. Girls might also need different or more individualized approaches to intervention to account for their strengths and needs. With increased awareness, knowledge and with improvements in screening and diagnostic methods, more autistic females are likely to be identified at a younger age, giving them the opportunity to benefit from early intervention (*2021-2023 IACC Strategic Plan for Autism Research*, 2023). Therefore, it is important that future studies, having a large number of females within the sample, investigate

whether being male or female may have a differential effect on the outcomes related to early intervention programs.

1.5 Structure of the thesis

The purpose of the following chapters is to expand and improve upon previous knowledge on early intervention research, unpacking "what works, for whom, for what and why" questions. Since there is no consensus on clinical-behavioral factors and no prior knowledge on biological characteristics which predict outcomes in the context of autism early intervention, it is important to investigate all factors outlined above and describe their effects on developmental change during treatment. Moreover, as several authors have suggested, autism is best described in terms of atypical development rather than a static entity (Chevallier et al., 2012; Dawson, 2008; Klintwall et al., 2015). However, to date, there are only a few large-scale intervention studies describing variability at the individual level with respect to time (Chen et al., 2022). Given that, our work is focused on developmental trajectories, which are a useful way to conceptualize the rate at which children learn during treatment (Klintwall et al., 2015).

With this aim, Chapter 2 describes our work conducted in collaboration with University of California, San Diego, where we investigated whether pre-treatment clinical and biological characteristics are able to predict individual developmental trajectories. Here we used gene expression patterns of blood leukocytes as possible biological predictors. This choice is based on the growing literature that has shown blood leukocyte gene expression activity may be a good proxy for assessing brain-relevant biological mechanisms and may be associated with different phenotypes in autism. (Gazestani et al., 2019; Lombardo et al., 2018). Therefore, by incorporating gene expression measures into our study design, we examined whether they were informative of children's developmental trajectories during treatment and which brain-relevant mechanisms were associated. For this investigation we selected a cohort of 41 autistic toddlers who all received the same standardized intervention at a very young age and provided

a blood sample and clinical-behavioral measures before the start of treatment. We computed individual treatment slopes based on the rate of children learning skills and we evaluated how well pre-treatment clinical-behavioral measures may be predictive of individual treatment slopes, in contrast to blood leukocyte gene expression features. To our knowledge this is the first time that is investigated whether and how information at the genomic level can predict treatment outcome. Results are described in Chapter 2, where a full description of findings is provided.

Given the current lack of agreement in the literature, we then focused our attention exclusively on the most studied putative predictors in early intervention research in order to answer to primary questions – such as, *what works, for whom*, and *for what*. To better answer the "for whom" question we conducted an individual participant data meta-analysis (IPD-MA) or 'mega-analysis' (Eisenhauer, 2021) of a large international early intervention consortium. In contrast to meta-analyses, this is an alternative approach which allows the isolation of individual factors to explore their key role in moderating children's developmental trajectories throughout the course of early intervention. In this work we included 11 international datasets, with longitudinal data on early intervention, comprising 645 autistic toddlers between 12 and 60 months of age at treatment start. Participants received two types of interventions either Early Start Denver Model (ESDM) or other treatment as usual/community (COM/TAU) interventions, with variable levels of intensity. Longitudinal data, common to most datasets, such as measures of cognitive skills, adaptive behavior abilities and autism symptom severity were used as treatment outcomes. Here, we examined the influence of a variety of treatment-specific factors such as type and intensity of treatment, as well as child's characteristics at the baseline, such as age, cognitive level and biological sex, for predicting developmental trajectories during treatment.

Finally, Chapter 4 briefly summarizes the findings of the thesis and discuss some of limitations, as well as potential future directions.

Chapter 2: Pre-treatment clinical and gene expression patterns predict developmental change in early intervention in autism.

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2.1 Introduction

Early detection and intervention in autism are topics of paramount importance because of the enormous potential to capitalize on the brain's enhanced plasticity during early development as a mechanism to positively impact outcomes (Dawson, 2008). While it is becoming increasingly clear that the biology of autism starts in early prenatal development (Courchesne et al., 2019, 2020) and that early behavioral signs begin to manifest before 18 months (Miller et al., 2017; Pierce et al., 2011), the mean age of diagnosis is still lagging far behind at 3–4 years of age (Baio et al., 2018; Maenner et al., 2020). In contrast to this reality, we have recently shown that diagnostic stability at much earlier ages is indeed high (Pierce et al., 2019), and thus the ability to detect and start

treatment earlier is feasible (Pierce et al., 2021). Some have suggested that detection and intervention before 24 months are key in order to capitalize on early neuroplasticity to facilitate optimal outcomes (Pierce et al., 2021; Webb et al., 2014). The impact of starting intervention earlier would likely be more total positive gains for the child (indexed by the absolute level of improvement). However, a less obvious, but perhaps equally important effect of earlier intervention could be a decrease in the variability of treatment responses at a group level. If this were the case, the reduction in treatment response variability might allow for more precise predictions about treatment outcomes. Understanding the ingredients that moderate and predict early intervention treatment response is of the utmost importance, especially given the current state of the field, where there is notably large heterogeneity in how children may respond to treatment (Vivanti, Prior, et al., 2014, p. 201). While the field has noted that some early interventions have an impact at a group level (French & Kennedy, 2018; Zwaigenbaum et al., 2015), what is less clear is how to predict an individual's specific response to the treatment and how to make that prediction before treatment begins. Understanding individual-level predictors of treatment response, particularly pre-treatment individual characteristics, is a key objective for precision medicine (Collins & Varmus, 2015; Lombardo, Lai, et al., 2019) applied to autism. Ideally, we would like to know what child specific characteristics are present before an intervention starts, in order to help us optimally predict how that specific intervention may affect the child. There are indications that some pre-treatment characteristics such as level of play, language, social cognitive abilities, IQ, autism symptom severity, and adaptive behavior may be important for moderating treatment response (Contaldo et al., 2020; Kasari et al., 2012; Landa, 2018; Rogers et al., 2012; T. Smith et al., 2015a; Vivanti et al., 2013; D. Zachor & Ben-Itzchak, 2017). In contrast to the many clinical studies that have been carried out on these phenotypic characteristics, biological predictors of treatment responses. remain largely unknown, leaving open the possibility that individual intrinsic biological characteristics of a child

may also moderate their response to treatment. If we better understood such treatment-relevant and individualized biological characteristics, this might yield unique insights into how and why some treatments work better for some children, but not others. In this work, we examine the effect of relatively early (<24 months) versus later (≥24 months) treatment start and how this may affect total gains and variability in treatment response. We also investigate whether pre-treatment standardized clinical behavioral measures and blood leukocyte gene expression patterns moderate how quickly a child will respond to early intervention. We operationalize treatment response here as the rate at which children respond over time and will refer to this concept from here on as "treatment slopes." Blood leukocyte gene expression offers up a powerful in-vivo alternative to clinical behavioral measures, as it helps to map out biological mechanisms of brain relevance but in a peripheral non-neural tissue. While the brain is largely an inaccessible tissue to assay mechanisms like gene expression in living patients, blood leukocyte gene expression has revealed a number of interesting brain-relevant characteristics that can be related to different phenotypes in living patients. Leukocyte expression patterns can be used in a classifier to predict diagnostic status (Pramparo, Pierce, et al., 2015), correlate with total brain size (Pramparo, Lombardo, et al., 2015), and are related to large-scale functional neural system response to speech (Lombardo et al., 2018), the patterning of thickness and surface area in the cerebral cortex (Lombardo, Eyler, et al., 2021), and social symptom severity (Gazestani et al., 2019). Differentially expressed genes in blood leukocytes are part of extended gene networks that are linked to highly penetrant autism-related mutations (Gazestani et al., 2019). Another revelation is that blood leukocyte genes associated with autism tend to be within a class of broadly expressed genes that are highly expressed in the brain and many other tissues (Lombardo et al., 2018). Broadly expressed genes are one class of important autism-associated genes that primarily have peak levels of expression during prenatal development (Courchesne et al., 2020; Gazestani et al., 2020). Given the sensitivity of blood leukocyte gene expression activity as a tool

for assessing the living biology behind autistic toddlers (Courchesne et al., 2019), we reasoned that there may be pre-treatment gene expression patterns in autistic toddlers that may be predictive of treatment slopes. In this study, we used the least absolute shrinkage and selection operator (LASSO) regression (Tibshirani, 1996) to model how clinical behavioral measures or gene expression patterns may be predictive of treatment slopes. LASSO is an important modeling strategy here for its use of L1 regularization, which acts to penalize largely uninformative features and results in a sparse solution that allows the user to isolate the specific subset of features that are highly predictive. To better understand treatment-relevant genes, we ran further analyses to test if these genes highly interact at the protein level, whether they overlap with known autism-related genomic and epigenomic mechanisms, and how they are expressed spatially throughout the brain.

2.2 Materials and Methods

Participants

This study was approved by the Institutional Review Board at the University of California, San Diego. Participants and families in this study were recruited as part of a larger multidisciplinary research project examining early neurobiological features and development of autism at the University of California, San Diego. Toddlers with a high likelihood for an autism diagnosis were identified from one of two sources: general community referral (e.g., website or outside agency) and a population-based screening method called Get SET Early (Pierce et al., 2011, 2021). Using this population-based screening approach, toddlers with a high likelihood for an autism diagnosis as young as 12 months were identified in pediatric offices with a broadband screening instrument—the Communication and Symbolic Behavior Scales-Developmental Profile Infant Toddler Checklist (Wetherby & Prizant, 2001).

Toddlers were evaluated and tracked every 6 months until their third birthday when a final diagnosis was given. Licensed clinicians with expertise evaluating and diagnosing autistic toddlers made final diagnoses based on clinical judgment and by incorporating criteria for autism on the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000; Pierce et al., 2019). Toddlers who were determined to be high likelihood for autism were offered intervention through our UCSD treatment program. Seventy-two families were referred for intervention, and 49 families chose to receive treatment in our program. Of the 49 children who received treatment from our program, 41 children (33 males, 8 females, mean age at the start of treatment = 22.77 months, SD age= 4.08, range= 13–27 months) also had a blood sample taken before the start of treatment and were therefore included in analyses for this work. Additional participant pre-treatment clinical information can be found in Table 1. Data from this study have been previously reported in Bacon et al. (2014), although this prior paper only focused on treatment and clinical behavioral data and did not examine gene expression.

Early intervention program

In order to reduce confounds that could be associated with differences associated with treatment type and administration, all toddlers received the same in-home treatment program using the Strategies for Teaching Based on Autism Research (STAR) curriculum (Arick et al., 2015). The STAR program is a comprehensive behavioral intervention program with a curriculum designed specifically for autistic children and includes instructional strategies of Discrete Trial Training (Lovaas, 1987a; Maurice et al., 1996; T. Smith, 2001), Pivotal Response Training (Koegel et al., 1987; Koegel, Robert L., 1988), and teaching in Functional Routines (Brown et al., 1987; McClannahan & Krantz, 2010). In an

effort to improve the developmental appropriateness of the curriculum for these very young children, the STAR curriculum was augmented with developmental approaches applied through Project ImPACT. Project ImPACT is a manualized curriculum developed by Ingersoll and Dvortcsak (Ingersoll & Dvortcsak, 2010) used to target social-communication goals in young autistic children. Project ImPACT focuses on the relationship between adult responsivity and children's social-communicative development. In the Project ImPACT curriculum, an early childhood interventionist (ECI) combines naturalistic behavioral strategies and developmental strategies. For example, the interventionist would respond to all communicative attempts by the child as if they were purposeful and recast expanded communication to facilitate communicative growth.

Treatment delivery

Each child received 6–12 hours per week (mean = 9.01, SD =1.53) of direct one-on-one intervention with a trained ECI at home until 36 months of age. ECIs were bachelor's degree or undergraduate-level research assistants with previous experience with young autistic children. Each ECI received extensive didactic and hand son training in behavioral principles and the STAR and Project ImPACT programs discussed above. Fidelity of implementation was reached for each intervention strategy as determined by using all components of the intervention correctly at least 80% of the time across two different children and monitored for maintenance. Programs were developed and supervised by master's degree-level clinicians (i.e., in-home coordinators) experienced in autism, with oversight from two doctorate-level clinical psychologists with extensive experience in early behavioral intervention for this population. In addition, parent coaching was provided throughout the course of participation.

Treatment outcome measure

The Adapted Student Learning Profile (aSLP) is a curriculum-based assessment for determining student learning goals and was adapted from the STAR curriculum to include additional goals from the Project ImPACT curriculum (see (Arick et al., 2015; Ingersoll & Dvortcsak, 2010)). The aSLP provides an extensive list of skills targeted in the STAR and Project ImPACT curricula and allows for the assessor to indicate the child's performance level on each skill across six domains: receptive language, expressive language, spontaneous language, functional routines, pre-academic concepts, and play and social interaction concepts. Data were analyzed using total aSLP scores across all domains rather than separate domain aSLP scores. The aSLP is administered by presenting each item up to five times to the child and observing the child's response. This is conducted in a structured format, and no teaching occurs during the assessment. The assessor then rates the child's response, indicating if the child did not demonstrate the skill or showed a partial demonstration of the skill or mastery of the skill. The entire aSLP takes ~30–45 minutes to complete. Each child's in-home coordinator completed an aSLP at intake and every 3 months thereafter to determine performance and progress. A child's performance was estimated by the subject-specific slope estimated in a linear mixed-effect model for modeling on the longitudinal aSLP scores (see section on "Developmental trajectory analyses").

Pre-treatment clinical behavioral measures

Pre-treatment clinical behavioral measures were collected to characterize the sample and utilized for analyzing how predictive such pre-treatment clinical measures (measured at treatment start) were of subsequent treatment slopes. The clinical measures analyzed were the Mullen Scales of Early Learning (MSEL), the

Vineland Adaptive Behavior Scales (2nd edition; VABS), and the ADOS. The MSEL assesses the developmental functioning of children between birth and 68 months (Mullen E. M. & American Guidance Service, 1995). An examiner measures child functioning level through a series of play-like tasks over five domains: gross motor, fine motor, receptive language, expressive language, and visual reception skills. For each scale, the assessment derives a T-score with a mean of 50 and standard deviation of 10, a percentile score, and an age equivalent score indicating at what developmental age the child is performing. An early learning composite (ELC) score is calculated from the total of scores on all scales (with the exception of the gross motor scale) with a mean of 100 and standard deviation of 15. The VABS provides a measure of adaptive skills used to cope with challenges of daily living (Sparrow, Sara S. et al., 2005). A caregiver completes a questionnaire regarding the individual's current level of functioning across five domains: communication, daily living skills, socialization, motor skills, and maladaptive behavior. All scales use standard scores with a mean of 100 and a standard deviation of 15, a percentile score, and an age equivalent score indicating at what developmental age the individual is performing. Scores on all scales are combined to obtain an overall adaptive behavior composite (ABC) with a mean of 100 and a standard deviation of 15.

Developmental trajectory analyses

To estimate aSLP trajectories for each toddler, we used a linear mixedeffect model to estimate longitudinal subject-specific intercepts and slopes as random effects. The subject-specific slopes (from here on called "treatment slopes") estimated from this model were extracted and used as the primary treatment outcome measure to be predicted by pre-treatment gene expression or clinical measures. These analyses were computed using the lme function from the nlme library in R. To better understand the effects of age at treatment start, we

used 24 months as the cutoff point for distinguishing very early versus later treatment start. This very early (<24 months) versus later distinction at 24 months (≥24 months) was made given that it is considered that the first 24 months of life are the critical early window for when early intervention could have the most impact (T. Smith et al., 2015a; Webb et al., 2014). Early (<24 months) versus later (≥24 months) start groups were not different with regard to treatment intensity (i.e., average number of hours per week in treatment; $F(1,39) = 0.004$, $p = 0.94$; <24 months mean =8.91, SD= 1.65; ≥24 months mean= 9.14, SD = 1.43) or with regard to general pretreatment developmental ability (i.e., pretreatment Mullen Early Learning Composite; Welch's $t(36.21) = -1.19$, $p = 0.23$; <24 months mean= 76.50, SD = 14.29; ≥24 months mean= 70.74, SD= 16.25). Linear mixed-effect models were used to examine differences in the aSLP as a function of very early (<24 months) versus later (≥24 months) start group. The linear mixed effect model included treatment start group (Early, <24 months of age at the start of treatment; Later, ≥24 months of age at the start of treatment), age, the interaction between age and treatment start group, number of days in treatment, treatment intensity (average number of hours per week), and pre-treatment Mullen Early Learning Composite as fixed effects and subject-specific age slopes and intercepts as random effects. We also investigated how variability in treatment slopes may differ between very early versus later start groups by computing the standard deviation of treatment slopes within each group and then quantifying the difference in standard deviation, computed as the difference score between later versus very early start groups. To test the standard deviation difference between groups against the null hypothesis of no difference in standard deviation difference score, we computed standard deviation difference scores over 10,000 random permutations of the very early or later start group labels, to derive a null distribution of standard deviation difference scores. A p value was then computed as the percentage of times under the null distribution that a standard deviation difference score was greater than or equal to the actual standard deviation difference score.

Blood sample collection, RNA extraction, quality control, and sample preparation

Four to six milliliters of blood were collected into EDTA-coated tubes from toddlers on visits when they had no fever, cold, flu, infections or other illnesses, or use of medications for illnesses 72 hours prior to blood draw. Temperature was also taken at the time of blood draw. Blood samples were passed over a LeukoLOCK filter (Ambion, Austin, TX, USA) to capture and stabilize leukocytes and immediately placed in a −20 °C freezer. Total RNA was extracted following standard procedures and manufacturer's instructions (Ambion, Austin, TX, USA). LeukoLOCK disks (Ambion, Cat #1933) were freed from RNA-later and Tri-reagent (Ambion, Cat #9738) was used to flush out the captured lymphocyte and lyse the cells. RNA was subsequently precipitated with ethanol and purified through washing and cartridge-based steps. The quality of messenger RNA samples was quantified by the RNA integrity number (RIN), with values of 7.0 or greater considered acceptable (Schroeder et al., 2006), and all processed RNA samples passed RIN quality control. Quantification of RNA was performed using Nanodrop (Thermo Scientific, Wilmington, DE, USA). Samples were prepped in 96-well plates at the concentration of 25 ng/µl.

Gene expression and data processing

RNA was assayed at Scripps Genomic Medicine (La Jolla, CA, USA) for labeling, hybridization, and scanning using the Illumina BeadChips pipeline (Illumina, San Diego, CA, USA) per the manufacturer's instruction. All arrays were scanned with the Illumina BeadArray Reader and read into Illumina GenomeStudio software (version 1.1.1). Raw data were exported from Illumina GenomeStudio, and data preprocessing was performed using the lumi package

(Du et al., 2008) for R (http://www.R-project.org) and Bioconductor (https://www.bioconductor.org) (Gentleman et al., 2004). Raw and normalized data are part of larger sets deposited in the Gene Expression Omnibus database (GSE42133; GSE111175).

Patient gene expression dataset

A larger primary dataset of blood leukocyte gene expression was available from 383 samples from 314 toddlers within the UC San Diego cohort, with the age range of 1–4 years old. The samples were assayed using the Illumina microarray platform in three batches. The datasets were combined by matching the Illumina Probe ID and probe nucleotide sequences. The final set included a total of 20,194 gene probes. Quality control analysis was performed to identify and remove 23 outlier samples from the dataset. Samples were marked as outliers if they showed low signal intensity (average signal two standard deviations lower than the overall mean), deviant pairwise correlations, deviant cumulative distributions, deviant multidimensional scaling plots, or poor hierarchical clustering, as described elsewhere (Pramparo, Lombardo, et al., 2015). This resulted in a final high-quality dataset that included 360 samples from 299 toddlers. High reproducibility was observed across technical replicates (mean Spearman's correlation of 0.97 and median of 0.98). Thus, we randomly removed one of each of the two technical replicates from the primary dataset. From the subjects in the larger primary dataset, a total of n = 41 also had treatment data; n = 36 from the Illumina HT12 platform along with n = 5 from the Illumina WG6 platform were used in this study. The 20,194 probes were quantile normalized and then variance filtered to leave the top 50% of highly varying probes (i.e., 10,097 probes). Treatment slopes were slightly different as a function of batch ($F(2,34) = 3.44$, $p = 0.047$), but were not associated with age at blood sampling ($F(1,34) = 0.0009$, $p = 0.97$), sex ($F(1,34) = 2.03$, $p = 0.16$), RIN ($F(1,34) = 0.22$, $p = 0.64$), or treatment intensity (average number of hours per week in treatment) ($F(1,34) =$

0.005, p = 0.93). Removal of variance associated with batch, sex, and RIN was achieved by using a linear model to estimate these effects in the training set of each cross-validation (CV) fold. This model computed on the training set was then applied to the test set for removing variance in such covariates.

Predictive modeling of treatment slopes

To predict individual differences in treatment slopes, we used a LASSO regression model (Tibshirani, 1996), which was used as predictors of either multivariate pre-treatment gene expression or clinical measures. LASSO uses L1 regularization (controlled by the lambda (λ) parameter) to shrink beta coefficients of uninformative features and thus reduce or effectively remove the influence of such features on the model. This feature is important for our purposes as we seek to compute a model that predicts treatment slopes but also informs us as to which features (e.g., genes or clinical measures) are most important for the model. For all LASSO modeling to assess the model's predictive utility, we used leave-one-out CV to partition the data into training and test sets. Within the training set, a 10-fold CV loop is used to estimate the optimal lambda parameter for the model. Cross-validated mean-squared error (MSE) and R2 were computed to evaluate the predictive value of the model. We also used permutation tests (1000 permutations) to randomly shuffle treatment slopes and construct a null distribution of MSE values under the null hypothesis. This null MSE distribution was used to compute a p value, defined as the proportion of times under the null distribution where an MSE value was as low or lower than the observed MSE value with unpermuted treatment slopes.

Protein–protein interaction analysis

The resulting gene list from the LASSO model predicting treatment slopes was then tested for evidence of protein–protein interactions (PPI). This analysis was achieved using the STRING database (https://string-db.org), with all parameters set to the STRING defaults (using all interaction sources and confidence interaction scores of 0.4 or higher). STRING also outputs enrichment results for Gene Ontology, Reactome, KEGG, and UniProt databases.

Autism-associated gene set enrichment analyses

To better link the set of treatment-relevant genes prioritized by the LASSO model, we tested this gene set for enrichment with other lists of genes known from the literature to be associated with autism. For autism associated genetic mutations, we used genes from SFARI Gene (https://gene.sfari.org) (Abrahams et al., 2013) in categories S, 1, 2, and 3 (October 2020 release). For genes with evidence of dysregulated expression in postmortem cortical tissue, we used differentially expressed gene lists from Gandal et al. (2018). At the epigenetic level, we also analyzed genes with evidence for differential histone acetylation in autism in postmortem prefrontal and temporal cortex tissue (Sun et al., 2016).

Spatial gene expression analyses

To get a better idea of the brain regions that are likely to be maximally affected by treatment-relevant genes prioritized by the LASSO model, we examined how these genes were spatially expressed across the brain using the Allen Institute Human Brain Atlas (Hawrylycz et al., 2012). Whole-brain gene expression maps for treatment-relevant genes were downloaded in the Montreal Neurological Institute space from https://neurosynth.org/genes/. These gene maps were then input into a whole-brain one-sample t test computed in SPM12

(https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Thresholding for multiple comparisons was achieved with voxel-wise false discovery rate (FDR) correction set to $q < 0.05$.

2.3 RESULTS

Differences between early versus late treatment start groups

A total of n = 41 toddlers were considered in all further analyses given that they had both gene expression and treatment data. In a first analysis, we examined whether individuals with a very early start to treatment (i.e., <24 months) would result in better outcomes than those who started treatment later (i.e., ≥24 months). As noted above, this early versus late distinction at 24 months was made given that it is considered that the first 24 months of life are the critical early window for when early intervention could have the most impact (T. Smith et al., 2015b; Webb et al., 2014). For this analysis, we used a linear mixed-effect model that modeled treatment start group (Early, <24 months of age at the start of treatment; Later, ≥24 months of age at the start of treatment), age, the interaction between age and treatment start group, number of days in treatment, treatment intensity (average number of hours per week), and pre-treatment Mullen Early Learning Composite as fixed effects and subject-specific age slopes and intercepts as random effects. Main effects were observed for age ($F = 134.09$, $p = 2.22e-16$) and treatment start group ($F = 20.92$, $p = 5.47e-5$), but there was no interaction between age and treatment start group ($F = 1.14$, $p = 0.28$). As shown in Figure 1A, the treatment start group effect is driven by the early group (<24 months) showing larger total treatment gains than those who start treatment relatively later (≥24 months) and that these effects cannot be explained by factors such as the duration of time in treatment, treatment intensity, or general pre-treatment developmental ability. However, the lack of an age-by-group interaction in

predicting treatment slopes indicates that there are no differences in the steepness of the trajectories between early versus late start groups. While the steepness of treatment slopes does not heavily differ on average between early versus late start groups, it is noteworthy that where the two groups do differ is on the variability in treatment slopes. Figure 1B, C shows a clear distinction between the late start group showing markedly more variable treatment slopes than the early start group. A permutation test further verified that the actual difference in standard deviations between late versus early start groups is highly significant relative to what this standard deviation difference would be under random group labeling ($p = 0.004$) (Figure 1D). This result indicates that while treatment slopes remain relatively consistent in their variability before 24 months, after 24 months the treatment slopes become much more variable.

Prediction of treatment slopes with pre-treatment clinical measures

We next examined if pre-treatment clinical behavioral measures could be predictive of treatment slopes. A LASSO model that included all pre-treatment ADOS, Mullen, and VABS subscales was able to significantly predict treatment slopes (mean MSE = 19.87, $p = 9.99e-4$, $R2 = 0.21$) (Figure 2A). Describing the correlations between treatment slopes and individual pre-treatment clinical measures, we find that all Vineland and Mullen subscales are significantly positively correlated, whereas total ADOS score and ADOS RRB were negatively correlated with treatment slopes (Figure 2B). These results are largely consistent with the idea from past work that pre-treatment clinical measures can be predictive of later treatment outcomes (Contaldo et al., 2020; Kasari et al., 2012; Landa, 2018; Rogers et al., 2012; T. Smith et al., 2015a; Vivanti et al., 2013; D. Zachor & Ben-Itzchak, 2017). However, as a new perspective on this effect, with longitudinal trajectories measured over more than just two time-points (e.g., pre-treatment and post-treatment), we find that pre-treatment clinical measures can

predict how steep an individual's treatment slope trajectory will be over the course of the treatment.

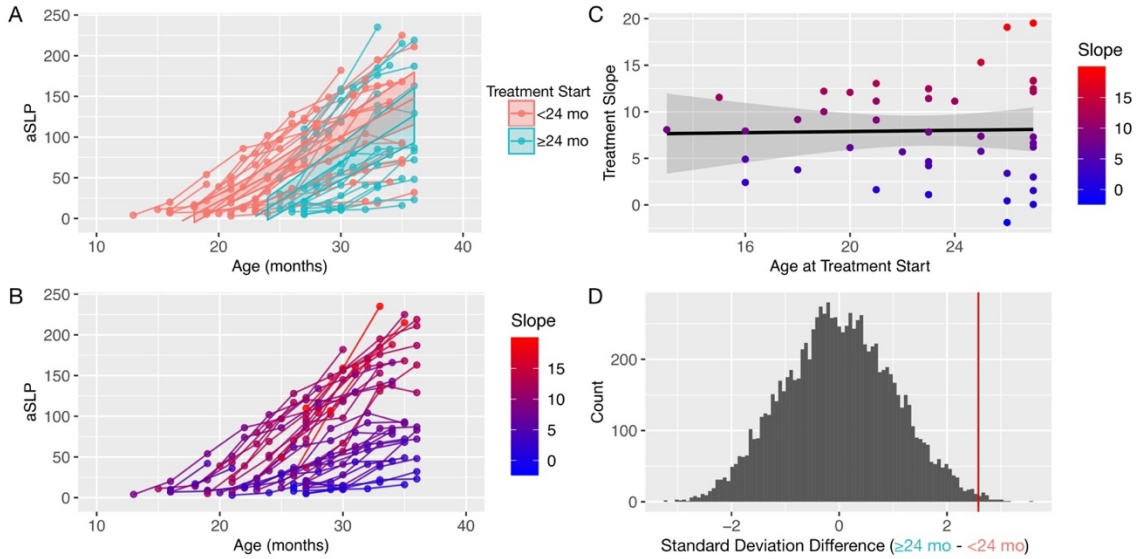

Figure 1. Treatment slopes and relationship with age at treatment start. *Panels **A** and **B** show trajectories of skill acquisition on the Adapted Student Learning Profile (aSLP) treatment outcome measure. Panel **A** shows these trajectories for treatment start groups defined by age at treatment start as either very early (<24 months, pink) or relatively later (≥24 months, turquoise). Panel B shows the trajectories but with each individual's data now colored by treatment slopes (colored from blue to red) estimated from a linear mixed effect model. Higher slopes indicate steeper trajectories and thus faster rate of skill growth over time whereas relatively lower slopes indicate less steep trajectories that can be interpreted as relatively slower rates of skill growth over time. Panel **C** shows treatment slopes for each individual as a function of age at treatment start (color indicates treatment slopes, as shown in panel **B**). Variability in treatment slopes becomes markedly larger when age of treatment start occurs after 24 months of age. Panel **D** shows a null distribution of difference in standard deviations over 10,000 permutations of random labelings of later (≥24 months) vs very early (<24 months) groups. The actual difference in standard deviation between later vs very early start groups is shown by the vertical red line.*

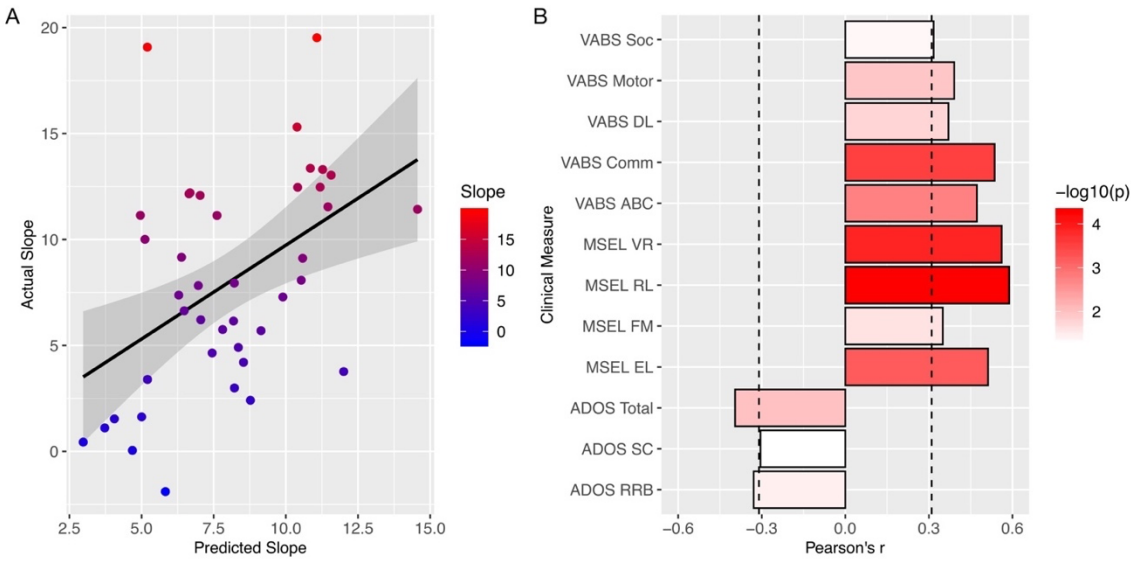

Figure 2. Predicting treatment slopes with pre-treatment clinical measures. *Panel **A** shows actual treatment slopes (y-axis) versus predicted treatment slopes from a LASSO model (x-axis) when using pre-treatment clinical measures as features. Color from blue to red indicates actual treatment slope values. Panel **B** shows the correlation (Pearson's r) between treatment slopes and each of the pre-treatment clinical measures. The coloring of the bars indicate the -log10(p-value) and bars that pass the vertical dotted line are measures that pass FDR q<0.05. Abbreviations: ADOS, Autism Diagnostic Observation Schedule; SC, social-communication; RRB, restricted repetitive behaviors; MSEL, Mullen Scales of Early Learning; VR, visual reception; FM, fine motor; RL, receptive language; EL, expressive language; VABS, Vineland Adaptive Behavior Scales; Comm, communication; DL, daily living skills; Soc, socialization; ABC, adaptive behavior composite.*

Prediction of treatment slopes with pre-treatment blood leukocyte gene expression data

We next asked if pre-treatment biological characteristics such as multivariate pre-treatment gene expression in blood leukocytes could also predict treatment slopes. Using a similar LASSO regression approach, we find that pre-treatment gene expression can also significantly predict treatment slopes (MSE = 21.67, p = 0.001, R2 = 0.13) (Figure 3A), albeit to a lesser extent than pretreatment clinical behavioral variables (e.g., 13% variance predicted with gene expression

versus 21% variance predicted with clinical behavioral measures). Next, we investigated which genes were most important in helping the LASSO model make such treatment slope predictions. Because LASSO uses L1 regularization to shrink coefficients of features that are less informative to 0, this allowed us to identify the subset of key genes that contribute to the model's predictive accuracy. Here we find that LASSO prioritizes 295 genes that help predict treatment slopes. Rather than being a random array of genes, these treatment-relevant genes show evidence of interactions at the protein level, as evinced with a PPI analysis (observed edges = 353, expected edges 306, p = 0.004) (Figure 3C). Further annotation of this treatment-relevant gene set was done with gene set enrichment analysis. This analysis discovered enriched biological processes such as regulation of protein localization and vesicle-mediated transport. Cellular compartments such as cytosol, intracellular organelle lumen, and cytoplasm were also enriched. With UniProt, we also discovered acetylation as a keyword enrichment (Figure 3C) (Table 2). Thus, treatment-relevant genes discovered by LASSO likely interact at the protein level and may be involved in processes such as protein localization, vesicle-mediated transport, and acetylation.

We next asked if this list of treatment-relevant genes might be associated with genetic mutations associated with autism or with genes that show dysregulated expression or histone acetylation in postmortem cortical tissue. Using gene lists from SFARI Gene (Abrahams et al., 2013) as well as a list of differentially expressed genes from Gandal et al. (2018), we find no evidence of enrichment in either of these lists. However, we did find the presence of four genes that are either high-confidence and/or syndromic autism genes in SFARI Gene— KMT2C, CORO1A, FBXO11, and PPP2R5D. KMT2C is noted as a rare de novo loss-of-function variant associated with autism (C Yuen et al., 2017; Iossifov et al., 2014; Krumm et al., 2015; O'Roak et al., 2012; Satterstrom et al., 2020; The DDD Study et al., 2014). CORO1A is a rare de novo loss-of-function variant associated with autism (Satterstrom et al., 2020) and is located within the

well-known autism-associated CNV region of 16p11.2 (Weiss et al., 2008) FBXO11 is another rare de novo loss of-function and missense variant in autism (Iossifov et al., 2014; Krumm et al., 2015) and appears in the autism-associated CNV region of 2p16.3 (Pinto et al., 2010; The Autism Genome Project Consortium, 2007). PPP2R5D is a known syndromic cause of autism and rare de novo loss-of-function variant associated with autism (Satterstrom et al., 2020; Shang et al., 2016). Each of these genes is a member of the PPI network shown in Figure 3C. Related to the UniProt enrichment in acetylation, we also found significant enrichment with genes that are differentially acetylated in autism postmortem cortical tissue (Figure 3B and Table 3). Specifically, treatment-relevant genes were enriched for upregulated histone acetylated genes in the prefrontal cortex tissue, but downregulated histone acetylated genes in the temporal cortex. This difference in spatial regions and directionality of the histone acetylation effect could suggest that these treatment-relevant genes may asymmetrically impact differing brain regions. Thus, while these treatment-relevant genes map onto a few genes with known evidence for high-confidence mutations or dysregulated gene expression, they are more strongly linked to genes that show evidence of differential histone acetylation in autism cortical tissue. This potentially indicates that treatment-relevant biology may be linked to epigenetic changes such as histone acetylation in cortical tissue. Given that early intervention intends to change behavior through reshaping the underlying biology, these links to histone acetylation could potentially provide key novel evidence as to how treatment effects may be moderated by individual molecular characteristics intrinsic to each individual.

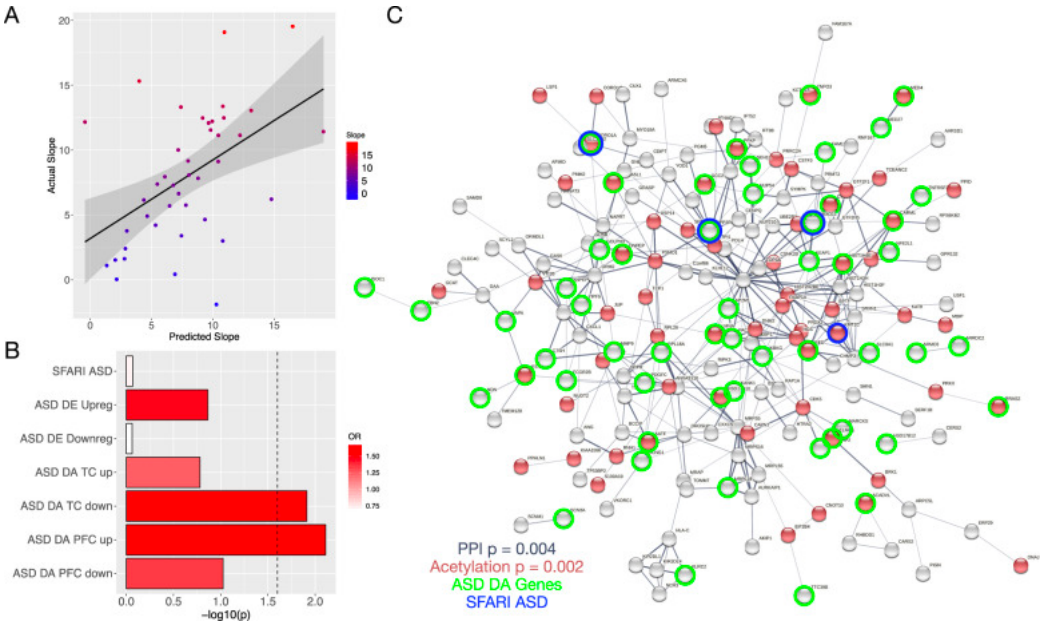

Figure 3. Predicting treatment slopes with pre-treatment blood leukocyte gene expression. *Panel **A** shows actual treatment slopes (y-axis) versus predicted treatment slopes from a LASSO model (x-axis) using pre-treatment blood leukocyte gene expression as features. The color from blue to red indicates actual treatment slope values. Panel **B** shows the −log 10 p value for the enrichment test (enrichment odds ratio (OR) colored in red) between treatment-relevant genes and ASD-associated gene lists. SFARI ASD refers to genes listed on SFARI Gene (https://gene.sfari.org), where mutations are known to be associated with ASD. DE Upreg or Downreg lists are genes that are (DE in postmortem cortical tissue (Gandal et al., 2018). ASD DA lists are genes whose histone proteins are DA in postmortem cortical tissue (Pinto et al., 2010). Bars passing the dotted line indicate gene lists that pass FDR q < 0.05. Panel **C** shows a graph of the protein–protein interaction (PPI) network of treatment-relevant genes from the LASSO model. Red nodes are genes enriched in UniProt for "acetylation." Green circles indicate genes whose histone proteins are DA in autism postmortem cortical tissue. Blue circles indicate genes that have high-confidence or syndromic ASD genes in SFARI Gene. DE differentially expressed, DA differentially acetylated, PFC prefrontal cortex, TC temporal cortex.*

Finally, we examined how treatment-relevant genes may be preferentially expressed in specific regions of the human brain. Leveraging spatial gene expression information from the Allen Institute Human Brain Gene Expression Atlas, we looked for which regions showed high levels of expression of these treatment relevant genes. To do this, we downloaded spatial gene expression maps for all 295 treatment-relevant genes from https://neurosynth.org/genes/ . With a

one-sample t test in SPM12, we ran a whole-brain analysis to identify brain areas where expression levels were significantly different from 0, correcting for multiple comparisons at voxel-wise FDR $q < 0.05$ (Figure 4). Here we find that subcortical areas are highly prominent particularly the thalamus, striatum, and claustrum. Amongst cortical areas, the most prominent regions are the anterior, middle, and posterior cingulate cortex (ACC, MCC, PC), dorsal and ventral medial prefrontal cortex (dMPFC, vMPFC), dorsolateral prefrontal cortex, ventral premotor cortex (vPMC), somatomotor cortex (SMC), temporoparietal junction (TPJ), planum temporale (PT), inferior parietal lobule (IPL), intraparietal sulcus (IPS), posterior superior temporal sulcus (pSTS), anterior temporal lobe (ATL), middle temporal gyrus (MTG), lateral occipital cortex, and insular cortex (Ins).

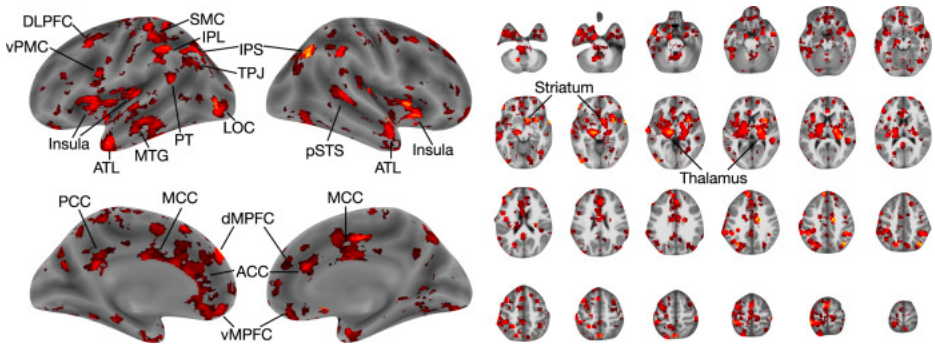

Figure 4. Regional gene expression in the brain for treatment-relevant genes*. This figure shows whole-brain analysis results (thresholded at $q < 0.05$ FDR correction for multiple comparisons) indicating which brain regions show high levels of expression for the treatment-relevant genes. Spatial gene expression was profiled here with the Allen Institute Human Brain Atlas. DLPFC dorsolateral prefrontal cortex, vMPFC ventromedial prefrontal cortex, dMPFC dorsomedial prefrontal cortex, ACC anterior cingulate cortex, MCC middle cingulate cortex, PCC posterior cingulate cortex, vPMC ventral premotor cortex, PT planum temporale, TPJ temporoparietal junction, SMC somatomotor cortex, IPL inferior parietal lobule, pSTS posterior superior temporal sulcus, ATL anterior temporal lobe, MTG middle temporal gyrus, LOC lateral occipital cortex.*

2.4 DISCUSSION

In this work, we examined whether pre-treatment clinical behavioral and blood leukocyte gene expression patterns could predict the rate of skill growth in response to early intervention in young toddlers with autism. Congruent with prior studies, pretreatment clinical behavioral characteristics such as language and communication and nonverbal cognitive ability are indeed helpful for predicting later treatment response (Contaldo et al., 2020; Kasari et al., 2012; Landa, 2018; Rogers et al., 2012; T. Smith et al., 2015a; Vivanti et al., 2013; D. Zachor & Ben-Itzchak, 2017), predicting ~21% of the variance in treatment slopes. A novel finding from this work is that pre-treatment gene expression patterns from blood leukocytes are also informative for predicting treatment slopes, predicting ~13% of the variance in treatment slopes. The effect of behavioral variables predicting more variance may not be surprising since such variables are conceptually and theoretically closer to what is being measured as the treatment outcome (e.g., behavioral change on the aSLP). However, the effect that pretreatment blood leukocyte gene expression can predict treatment slopes at some level is a revelation, given that prior to this work it was unknown whether pre-treatment biological factors such as blood leukocyte gene expression could predict treatment slopes at all. Examining the gene expression signal that is predictive of treatment slopes more closely, our LASSO modeling approach prioritizes a subset of 295 genes that highly interact at the protein level and which are enriched for biological processes such as acetylation. Expanding on the idea of acetylation as a treatment relevant biological process, we also discovered that these treatment-relevant genes are enriched for genes that are differentially histone acetylated in postmortem cortical tissue of autistic patients (Sun et al., 2016). Because our signature was revealed in blood and not in brain tissue and that the UniProt enrichment of acetylation is not necessarily brain-specific, the evidence that these genes also have a differential impact on histone acetylation in autism cortical tissue is an

important cross-tissue correspondence. Given that the central dogma behind the early intervention is to capitalize on an individual's heightened propensity for neurobiological plasticity and change in early development, these findings suggest that one key to predicting an individual's propensity for such change may be hidden within individualized and intrinsic biology related to histone acetylation. In other words, predicting early intervention treatment response may hinge critically on how susceptible an individual's intrinsic biology is to experience- or context-dependent control over the regulation of gene expression. This idea bodes well with general ideas regarding histone acetylation as one of the primary molecular influences over activity-dependent gene expression, which would then subsequently alter experience-dependent learning and memory processes (Gräff & Tsai, 2013) that are critical ingredients of early intervention. Because our signature was revealed in blood and not in the brain, we additionally tested how these genes are expressed spatially throughout human brain cortical tissue. If the signature was brain-irrelevant, we would not see high levels of expression within specific brain regions. Contrary to this null hypothesis, we discovered that treatment-relevant genes highly express throughout a range of subcortical and cortical areas. Subcortical areas such as the thalamus, striatum, and claustrum all have extensive connections to the various cortical areas implicated (Behrens et al., 2003; Choi et al., 2012; Cohen et al., 2009; Crick & Koch, 2005). The cortical areas fall within well-known large-scale circuits like the default mode, salience, and somatomotor network. The default mode network is noted for its overlap with regions considered integral for social brain circuitry (e.g., dMPFC, vMPFC, PCC, TPJ, ATL, pSTS) and social-communicative functions linked to the domains affected in autism (Buckner & DiNicola, 2019; Trakoshis et al., 2020). Other regions relevant to the mirror system are also apparent (e.g., vPMC, Ins, MCC, IPL, IPS, SMC) (Keysers et al., 2010; Keysers & Gazzola, 2007, 2009). Language-relevant regions are also notable, such as (e.g., PT, MTG, vPMC, ATL) (Friederici, 2012; Hickok & Poeppel, 2007). While speculative, this evidence could be suggestive of the possible impact of treatment-relevant genes on circuitry

that plays important roles in cognitive and behavioral domains targeted by early intervention and which are key domains of importance in the early development of autism. Overall, this result corresponds well with the earlier discussed brain-relevant enrichment in differential histone acetylation in autism cortical tissue, for indicating that these genes, although identified in blood, have an important brain-relevant impact. In addition, we also found that starting treatment before versus after 24 months is a meaningful distinction (T. Smith et al., 2015a; Webb et al., 2014). Toddlers who started treatment before 24 months showed larger overall gains than those starting treatment after 24 months, even when controlling for the amount of time in treatment, treatment intensity, and general pre-treatment developmental ability. This result is compatible with the main ideas behind why early intervention is crucial before 24 months (Dawson, 2008; Pierce et al., 2021; Webb et al., 2014). Also compatible with the idea that treatment start before versus after 24 months is important, we also discovered that treatment slopes are much more variable once past 24 months of age. The enhanced variability of treatment slopes after 24 months is important, as it underscores how heterogeneity can be magnified with a later treatment start. One implication of this result is that prediction of treatment outcome is a much more difficult task when the child begins treatment after 24 months of age. This is another consideration for why early detection and intervention is key—treatment outcomes tend to be more consistent if treatment begins before 24 months. There are some caveats and limitations that are necessary to address to interpret the present findings. First, this study is correlational in nature and does not contain a contrast group to compare the STAR program to. As such, interpretation of the effects here as related specifically to treatment-related learning cannot be disentangled from possible maturational effects. The effects here should be interpreted as a prediction of developmental change in the context of an early intervention, but should not be interpreted as predicting change that is specifically driven by elements of the intervention itself per se. Second, the results reported here are associated with a specific evidence-based early intervention program that contains a mixture of

elements from various programs (e.g., applied behavioral analysis, pivotal response training) and administered by highly trained providers with systematic probes of fidelity of implementation. The use of the same standardized treatment approach for all participants is a strength of the current study. However, given the variety of different types of early intervention programs available today (e.g., Early Start Denver Model, parent-mediated communication-focused treatment), caution must be taken in generalizing these findings to other intervention programs. A question for future work would be to examine whether these findings extend to other widely used early intervention programs. Third, the aSLP was not administered by blind assessors. In addition, as is true for most curriculum-based assessments, the aSLP is not a standardized psychometric instrument. However, treatment slopes as indexed by the aSLP were highly correlated with other psychometrically well-validated instruments such as the Mullen and Vineland that were administered by blind assessors. This indicates that aSLP has some construct validity for measuring developmental abilities despite these limitations. Fourth, the outcome measure operationalized as the rate of improvement over time is not a commonly used metric to evaluate early intervention response. In most designs, there are time-locked pre- and post-testing measures to evaluate treatment response. However, the rate of response to treatment from multiple longitudinal measurements may be a more sensitive measure of treatment response than a change score sampled at just two points in time. Fifth, while the sample size of this study is moderate to above average for what is typical in most treatment and gene expression studies (Ansel et al., 2017; Fuller et al., 2020), future work replicating these findings with larger samples is needed. Notably, given recommendations such as the use of a higher alpha threshold for statistical significance (e.g., $\alpha = 0.005$) (Benjamin et al., 2017) for the discovery of novel effects, all primary effects of interest reported here would still survive this more conservative alpha threshold. Another caveat here regarding sample size in gene expression studies is that studies of brain tissue are typically much smaller and deal with RNA quality that is much lower than what is typical in studies using

blood samples. In addition, brain tissue studies typically have much larger age ranges spanning toddlerhood to adulthood. Thus, there is much larger age-related heterogeneity that is apparent in brain tissue compared to blood. Combining these caveats regarding higher sample size, higher RNA quality, and less age-related heterogeneity suggests that the current study is typically ahead of the norms within the context of gene expression studies in autistic patients. Finally, future work could examine whether different approaches to merge multiple data modalities such as pre-treatment gene expression and clinical measures might help to better predict treatment slopes. In the current work, we did not investigate this possibility as it is beyond the scope of the current investigation and requires much more sophisticated approaches tailored specifically for multiple modality data fusion, especially in situations where different modalities are high dimensional and/or differ substantially in dimensionality (Sui et al., 2012; Wang et al., 2022). In conclusion, this work shows the importance of early treatment start ideally before 24 months and also shows for the first time that blood gene expression characteristics can predict how fast autistic toddlers respond to early treatment. While clinical behavioral variables outperformed gene expression measures, the signal within gene expression is important because it potentially indicates that a key biological ingredient for determining an individual's treatment outcome is susceptibility to epigenetic change via mechanisms such as acetylation. Understanding how this treatment-relevant biology affects neuroplasticity and experience-dependent learning is a key next step towards how such molecular mechanisms are linked to heterogeneous outcomes in autism.

Chapter 3: The effects of individual child characteristics and treatment related factors on developmental trajectories in autism: An individual participant data meta-analysis

This chapter is in preparation as: The effects of early intervention type and child individual factors on developmental trajectories in autism: An individual participant data meta-analysis. (*in prep.*) Michael V. Lombardo & Elena Maria Busuoli

3.1 Introduction

Autism is one of the most common neurodevelopmental conditions in society today and represents a broad population of individuals that are heterogeneous across multiple scales from biology to phenotype (Lombardo, Lai, et al., 2019), across development (Gentles et al., 2023) and important clinical outcomes (e.g., responses to treatment); (Godel et al., 2022; Lombardo, Busuoli, et al., 2021; Vivanti, Prior, et al., 2014). A top priority on the path towards precision medicine is the development of therapeutic/intervention approaches in early development that can facilitate positive outcomes aligned with multiple different stakeholder perspectives (Lombardo & Mandelli, 2022; Pellicano & Den Houting, 2022; Tager-Flusberg & Kasari, 2013). The push for earlier diagnosis and intervention is also paramount (Pierce et al., 2019; J. D. Smith et al., 2022) and predicated on the idea that there is higher probability of facilitating more positive outcomes with greater neural plasticity offered during the earliest periods of neurodevelopment (Dawson, 2008). The top priorities/questions for early

intervention research are quite clear. We seek to better understand *what works*, *for whom*, and *for what* (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). Since heterogeneity in responses to treatment is a high priority topic, it is important to further unpack the '*for whom*' question. We need to better understand *how* and *why* early intervention is facilitating differential individualized outcomes. For these important questions, we have to go beyond examination of on-average group-differences due to intervention and dissect what are the factors or characteristics present in children before treatment begins that would help us predict the subsequent developmental path during treatment (Lombardo, Busuoli, et al., 2021; Mandelli et al., 2023; Vivanti, Prior, et al., 2014) and what is changing in terms of underlying neurobiology as a function of the differential experience and learning provided by early intervention (Dawson et al., 2012; Lombardo, Busuoli, et al., 2021).

Prior reviews and meta-analyses of autism early intervention research provide an initial starting point on the '*what works*' and '*for what*' questions, since such work helps us get a better sense of how early intervention may or may not have effects at a group-level. The literature on early intervention is quite large, but also presents a mixed picture (Crank et al., 2021; Eldevik et al., 2010; Fuller et al., 2020; Howlin et al., 2009; McGlade et al., 2023; Rodgers et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020; Warren et al., 2011). Without filtering or controlling for study quality/bias, there is some evidence that various types of early intervention (e.g., developmental and naturalistic developmental behavioral interventions; NDBI) can be effective on-average in changing a variety of outcomes including language, intellectual and social communication abilities (Fuller et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Feldman, et al., 2020). However, a very different picture emerges when evaluating a smaller handful of high-quality randomized control trials (RCT).

With this particular restriction, early behavioral intervention may be limited in effectiveness in changing language or core autism symptom domains (Crank et al., 2021; McGlade et al., 2023; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020).

The mixed picture behind most recent meta-analytic evidence underscores the need for more high-quality RCT studies. However, there are also several limitations in sole reliance on meta-analytic inference. First, meta-analytic inferences are made to test whether replicable non-zero group-level effects exist in the literature. This goal is tailored to answer the 'what works' and 'for what' questions quite well. However, these goals are inherently different from the goals of decomposing heterogeneity in response to treatment (i.e. the 'for whom' question). If meta-analysis on a small number of high-quality RCTs suggests that on-average group-level effects are small or altogether absent (Crank et al., 2021; McGlade et al., 2023; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020), this does not preclude the fact that the intervention may still work considerably well for specific types of individuals. Thus, a different analytic approach is needed to better answer the 'for whom' question. In contrast to meta-analysis, approaches such as individual participant data meta-analysis (IPD-MA) or 'mega-analysis' (Eisenhauer, 2021) may be more well-suited. Mega-analysis utilizes the raw data from individual participants across many studies and allows for testing not only group-level effects, but also factors that explain individual differences in treatment outcomes. To our knowledge, the only IPD-MA/mega-analysis in the literature (Rodgers et al., 2021) compares early intensive ABA based interventions (such as NDBI and variants of EIBI) to treatment-as-usual (TAU) or eclectic interventions. The primary findings here suggest that early intensive ABA-based interventions have a small effect on intellectual and adaptive functioning. Other moderating individual difference factors such as sex, age, baseline cognitive or adaptive functioning level, were not associated with treatment outcomes.

A second limitation behind meta-analytic work is that statistical power to detect on-average group-level effects may be low when restrictions are placed on filtering for only a small subset of high-quality studies. These handful of studies themselves may also have relatively small sample sizes. In the case of small sample sizes, unadjusted descriptive statistics and standardized effects size computed from them will be inherently less precise and possibly more prone to effect size inflation (Lombardo, Lai, et al., 2019). Utilizing unadjusted statistics in meta-analysis is a necessary step to homogenize effect size computation across heterogeneous studies. However, this attribute does not allow for correction for covariates within-study that would typically be applied in each individual study. To tackle this issue to some extent, moderator analyses from meta-regression models can be implemented to test associations with study-specific factors and covariates (e.g., mean age at treatment start). These moderator analyses are limited though to study-specific summary factors (e.g., mean age) that themselves are descriptive statistics computed per study and are inherently not the same measuring and controlling for those covariates within individuals (e.g., age at treatment start per individual). Again, utilization of mega-analysis would get past this limitation by allowing for covariates to be applied within-study.

A third limitation behind restricting meta-analytic inference to only a small handful of high-quality RCTs is that RCT can be quite restrictive in how they sample the autism population (Rogers et al., 2019) and may not present a full generalizable picture for how early intervention works when deployed in community settings. For instance, while restrictive sampling employed in most recent RCTs of Early Start Denver Model (ESDM) tend to show no significant difference in treatment outcomes compared to TAU (Rogers et al., 2019), a wider meta-analysis of ESDM studies shows that under a broader range of studies that may be less restrictive shows more promising evidence for ESDM effectiveness (Fuller et al., 2020). Thus, a potentially more generalizable picture regarding early

intervention might be observed by accumulating raw data in mega-analysis across a wider range of intervention studies, which compose both more restrictive RCTs and less constrained interventions in community settings.

To overcome these limitations, we initiated the Autism Early Intervention Research (AEIR) consortium, to pool together many studies on early intervention in autism for the purpose of mega-analysis. AEIR has been able to pool together 645 autistic individuals with age range between 13 to 60 months receiving two treatment types: Early Start Denver Model (ESDM) and other types of early interventions that can be found in community settings as the typical treatment-as-usual (COM/TAU). Here we report results from a mega-analysis of AEIR consortium data to elucidate main effects and interactions between factors such as age, treatment type, treatment intensity, age at treatment start, and pre-treatment developmental level as possible factors that can predict significant variance in treatment responses.

3.2 Materials and Methods

All work with secondary data analysis reported here was approved by the Province of Trento Azienda Provinciale per i Servizi Sanitaria (APSS) ethical committee under protocol IIT EMN-755816-002-AUTISMS.

Datasets

Data utilized in this study come from 11 international datasets on early intervention in autistic toddlers as part of the Autism Early Intervention Research (AEIR) consortium. Many, though not all, of these datasets have already been previously published independently (Bacon et al., 2014; Colombi, 2017; Contaldo

et al., 2020; Godel et al., 2022; Muratori & Antonio Narzisi, 2014; Rogers et al., 2019; Sinai-Gavrilov et al., 2020; Vivanti et al., 2019; Vivanti, Paynter, et al., 2014). The final dataset comprises n=645 children with a diagnosis of autism (n=128 females, n=517 males), with ages at treatment start ranging from between 13-60 months. All datasets have at least 2 timepoints within an individual but some datasets may possess more than 2 timepoints per individual.

Early Intervention Programs

Participants received between 3-27 months of early intervention with different types of approaches and variable levels of treatment intensity. Given the considerable number of participants that completed one type of early intervention (e.g., ESDM; n=304, 65 females, 239 males) we partitioned the data into two groups - ESDM or non-ESDM treatment as the comparison group. The non-ESDM group is a group we refer to Community/Treatment-As-Usual (COMM/TAU; n=341, 63 females, 278 males) as it represents a combination of other types of early interventions commonly used in the literature and in community settings (e.g., speech therapy, occupational therapy, ABA/Discrete Trial Training (Lovaas, 1987b), Pivotal Response Training (Koegel, Robert L., 1988), etc.). Early interventions varied in their format of administration and could be comprised of individual, group, and parent intervention components.

Measures

All datasets had commonalities with reference to measures utilized to examine cognitive ability, language, motor, adaptive functioning, and autism symptom severity. For cognitive ability, language and motor skills, most datasets in AEIR except those originating in Italy, utilized the Mullen Early Scales of Learning (MSEL) (Mullen E. M. & American Guidance Service, 1995). Scores on the MSEL that were utilized were subscales of expressive and receptive language

(EL, RL), visual reception skills (VR) and fine motor (FM). For these subscales, we utilized age-equivalent scores in our developmental trajectory analyses to examine how outcome measures change over age and scale with changes in age-equivalent score growth. To examine pre-treatment developmental level on the MSEL, we used the Early Learning Composite score, which is a standardized score with a mean of 100 and standard deviation of 15. Since datasets from Italy did not utilize the MSEL (because of lack of translation into Italian), Italian datasets were excluded for all models where MSEL was the dependent variable. However, in the case where VABS or ADOS scores were utilized as the dependent variable, then Italian datasets were included. In this situation, pre-treatment developmental level was needed, and here we utilized developmental quotient score on the Griffith Mental Development Scales (GMDS) (Griffiths, 1970). Adaptive functioning was measured in all datasets with the Vineland Adaptive Behavior Scales (VABS) (Sparrow, Sara S. et al., 2005). For the VABS we utilized standardized scores on domains such as Communication, Socialization, Daily Living Skills, and Motor. Finally, with regard to autism symptom severity, here we utilized the Autism Diagnostic Observation Schedule (ADOS) Calibrated Severity Scores (CSS) (Lord et al., 2000). ADOS CSS scores measure severity as a total across social-communication and restricted repetitive behavior domains. ADOS CSS scores are advantageous because they are standardized relative to a child's age and language ability and ensure comparability across ADOS administration modules.

Pre-treatment characteristics

Child's characteristics at treatment start were collected and utilized for analyzing whether they are associated with treatment outcomes. With this aim were selected clinical measures, demographic and intervention specific factors, on the basis of the most relevant research findings described in previous literature (Eapen & Crncec, 2016; Sinai-Gavrilov et al., 2020; Vivanti et al., 2019; Vivanti,

Prior, et al., 2014; Warren et al., 2011; D. Zachor & Ben-Itzchak, 2017). We analyzed cognitive abilities at treatment start reported as developmental quotient scores (DQ), taken from MSEL early learning composite (ELC) scores or GMDS developmental quotient scores. Furthermore, age at treatment start and biological sex were selected and analyzed as demographic factors that might be associated with treatment outcomes. In this study, the number of females in the sample allowed us to test whether there is sex treatment related difference. Also, treatment-specific factors potentially able to predict treatment outcome measures were assessed, such as intensity and type of early intervention (ESDM or comparison group). Intensity index of intervention was computed for each child, following the formula: (weeks of intervention * hours per week * number of adults / number of children present) (Waizbard-Bartov et al., 2021).

Statistical Analyses

All analyses utilized throughout are linear mixed effect models to handle repeat measures, assess developmental trajectories, and handle nested within-study factors. Three sets of models were analyzed here organized according to MSEL, VABS, and ADOS. For MSEL models, the dependent variable was always age-equivalent scores. We presented 4 MSEL models, one per each MSEL subscale. For VABS models, the dependent variable was always standardized domain scores, with one model per each VABS domain. One model was utilized for the ADOS and had total CSS scores as the dependent variable. In terms of the independent variables, we used the same independent variables for all models. Fixed effects in the models were always age, treatment type, treatment intensity, sex, age at treatment start, and pre-treatment developmental quotient. Interaction effects were also modeled as fixed effects, particularly, interactions between age, age at treatment start, and pre-treatment developmental quotient. Interactions with treatment type were also modeled, such as interactions with sex, age, age at

treatment start, and pre-treatment developmental quotient. Random effects were also utilized in the model to account for subject-specific age intercepts and slopes (i.e. age modeled within subject-ID). Dataset-ID was also utilized as a random effect with random intercept to capture the nested structure of specific datasets and the variance associated with each dataset. All linear mixed effect modeling was conducted in R using the *lmer* function within the *lmerTest* R library. To identify significant effects across multiple analyses, p-values were utilized to compute FDR values, and then FDR control for multiple comparisons was achieved with thresholding at $q<0.05$. To visualize differential rates of growth as a function of some other continuous variable (e.g. interactions between age and age at treatment start or pre-treatment developmental quotient), we split individuals into deciles based on pre-treatment scores and then plotted trajectories for each decile.

3.3 Results

In this mega-analysis, we report data compiled from the AEIR consortium across 11 datasets (Figure 5), and comprising n=645 autistic individuals split into two main types of early intervention treatments - ESDM (n=304, male = 79%, age range = 14-57 months) versus COM/TAU (n=341, male = 81%, age range = 13-60 months). See Table 4 for a breakdown of descriptive statistics of baseline measures between these treatment types. Treatment types did not differ in proportion of males versus females ($\chi^2 = 0.68$, $p = 0.40$). Treatment intensity was not different between treatment types ($F(1,631) = 0.04$, $p = 0.84$). Age at treatment start was marginally different ($F(1,631) = 3.81$, $p = 0.051$), with ESDM showing slightly younger ages than COM/TAU. Pre-treatment developmental quotient was slightly different ($F(1,561) = 4.78$, $p = 0.029$) with ESDM showing slightly higher scores than COM/TAU.

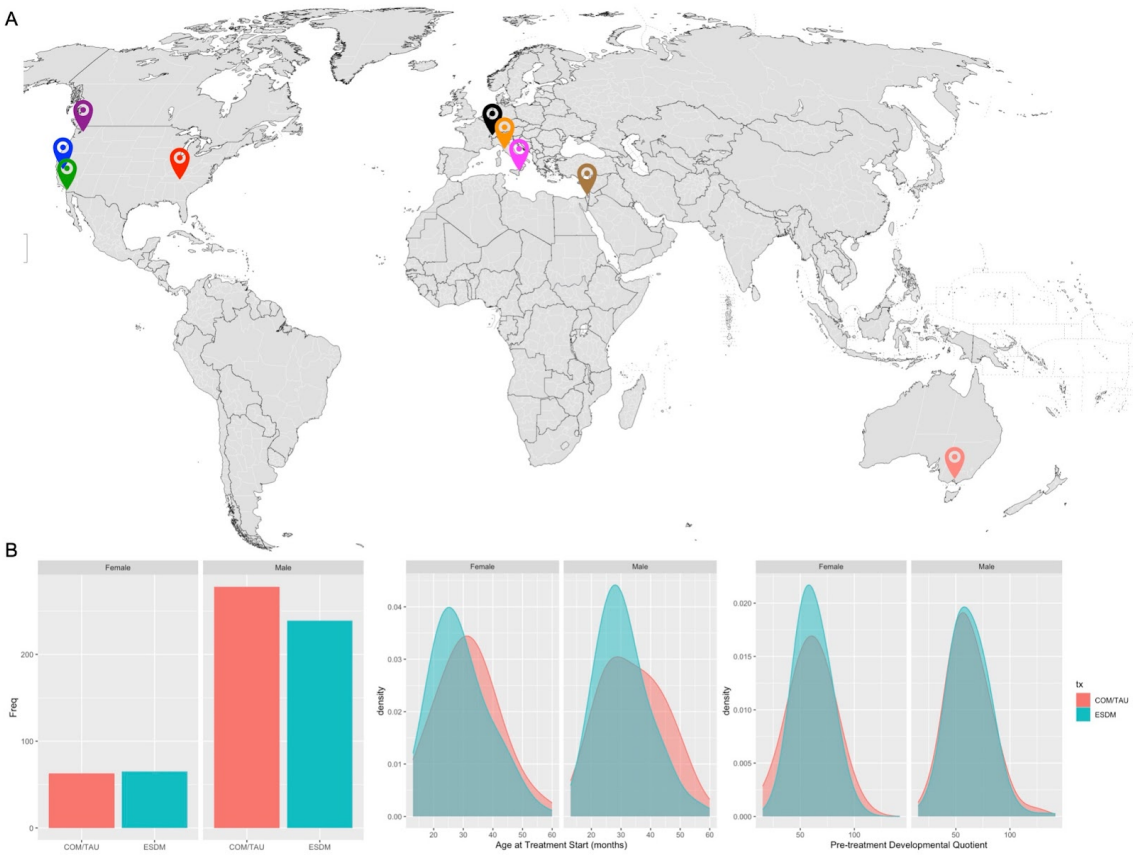

Figure 5: AEIR consortium. *Panel **A** shows sites around the world where data from the AEIR consortium originates from. On the left of panel **B** are sample sizes broken down by treatment type and sex. In the middle of panel **B** are density plots of age at treatment start. On the right of panel **B** are density plots of pre-treatment developmental quotient.*

Effects of treatment type and intensity

In our first set of analyses we examined the '*what works*' and '*for what*' questions specifically with regards to the contrast of ESDM versus COM/TAU treatments. Within the context of how we modeled effects of treatment type, we specifically look for interactions between treatment type and age. ESDM does seem to have a differential effect on developmental trajectories compared to COM/TAU, particularly for receptive language (RL; F = 8.49, p = 3.89e-3) and marginally for non-verbal cognitive skills (MSEL VR; F = 5.33, p = 0.0212).

These differences are driven by a steeper rate of growth for individuals in ESDM compared to COM/TAU (Figure 6). No interactions between treatment type and age were observed for other MSEL scales (EL, FM) or for VABS domains or ADOS CSS (see Table 5 for statistics). Thus, the effects of treatment type are relatively subtle, but demonstrate some evidence that ESDM may facilitate development of language and non-verbal cognitive skills over and above other kinds of commonly used early interventions used in the community. In contrast to the promise of some subtle effects of treatment type, analyses robustly showed no evidence for an effect of treatment intensity on any of the dependent variables (see Table 5 for statistics).

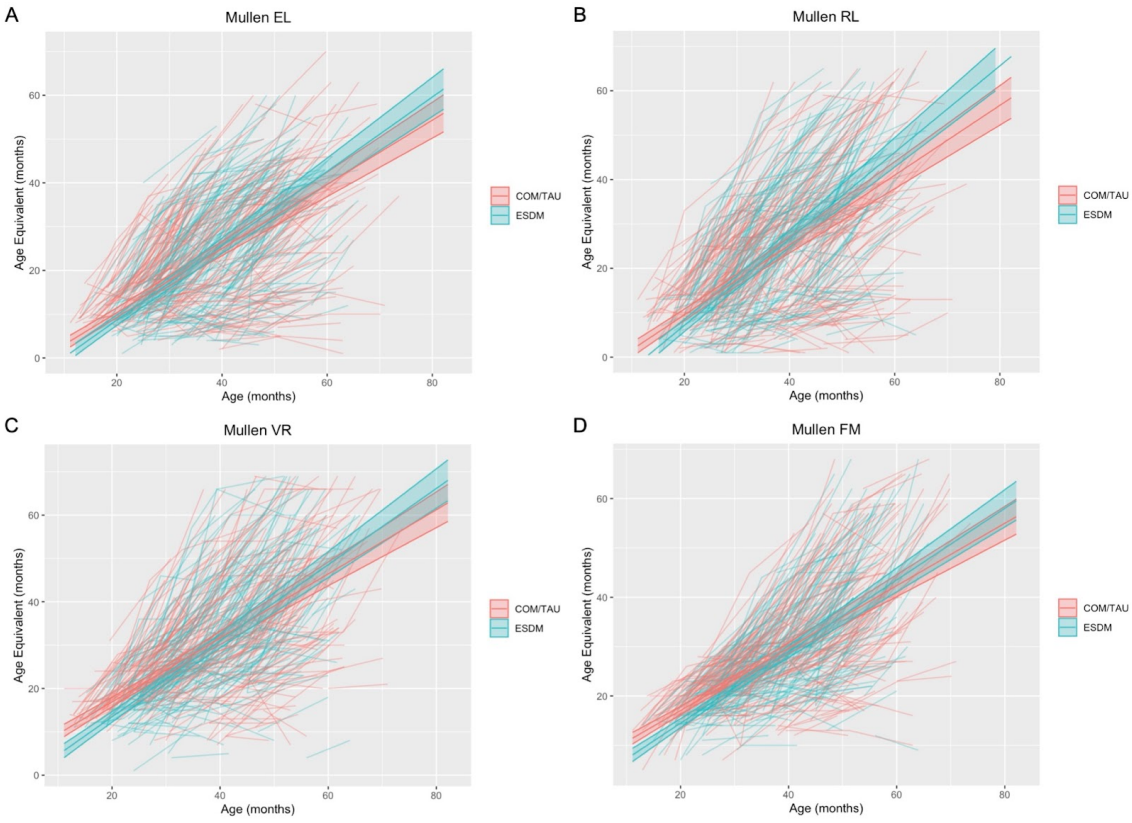

Figure 6: Comparisons of ESDM versus Community/Treatment-As-Usual (COM/TAU) interventions. *This figure shows spaghetti plots of MSEL subscales for ESDM (blue) compared to COM/TAU (pink). The x-axis shows age in months, while the y-axis shows age-equivalent scores in months. Individual trajectories are shown with transparent*

lines, while the group trajectory along with 95% confidence bands are shown overlaid on top. Subtle statistically significant differences exist for receptive language (RL) and visual reception (VR) subscales, while trends in the same direction are apparent particularly for expressive language (EL).

Ubiquitous and strong effects of age at treatment start and pre-treatment developmental quotient

Whereas effects of treatment type at a group-level are relatively subtle, there remains the fundamentally important question for precision medicine, which is '*for whom and how*' does early intervention work best for individuals? To evaluate this question, we tested individual-level variables such as sex, age at treatment start, and pre-treatment developmental quotient. Whereas no effects appeared for sex for MSEL, VABS, or ADOS, there were remarkably strong effects for age at treatment start (Figure 7) and pre-treatment developmental quotient (Figure 8). These effects were ubiquitous, in both intervention types, across all MSEL, VABS, and ADOS dependent variables examined, with the exception of the VABS Motor domain, which showed no effect of age at treatment start (see Table 5). The nature of all of these strong individualized-effects are described by better outcomes in those that start treatment earlier and for individuals who start treatment with higher developmental quotients.

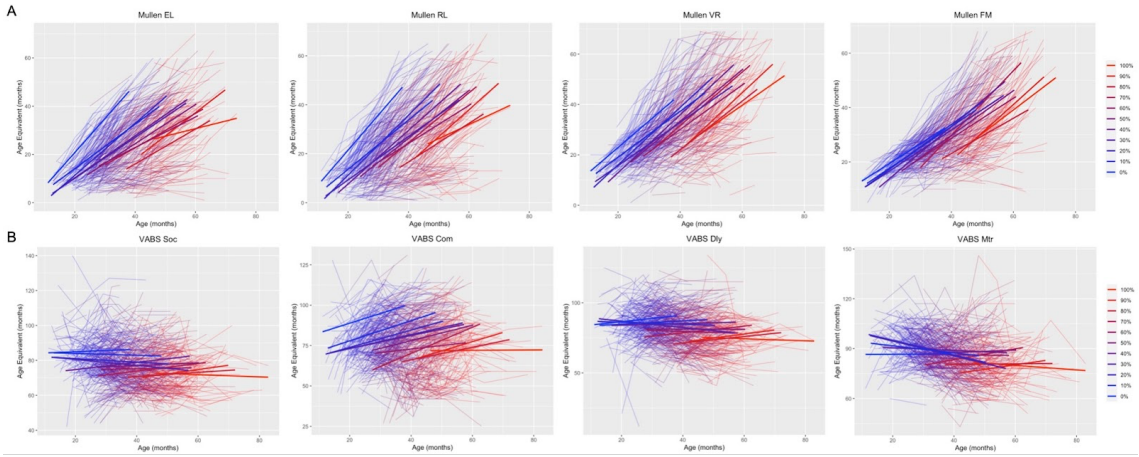

Figure 7: Effect of age at treatment start on MSEL and VABS scores. *This figure shows spaghetti plots of MSEL (**A**) and VABS (**B**) subscales, whereby color indicates deciles defined by age at treatment start (younger-to-older deciles follow blue-to-red color change). The x-axis shows age in months, while the y-axis shows age-equivalent scores in months. Individual trajectories are shown with transparent lines, while the decile group trajectories are shown overlaid with thicker lines.*

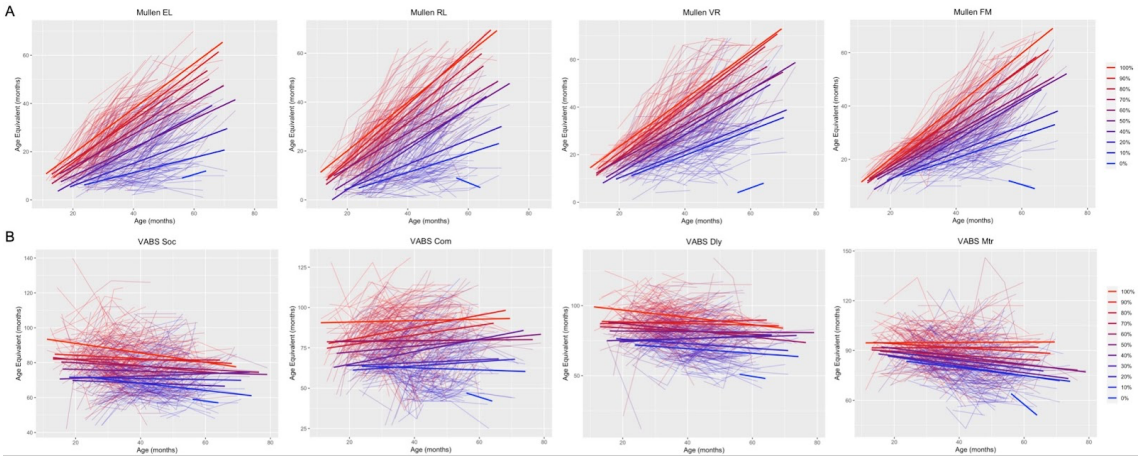

Figure 8: Effect of pre-treatment developmental quotient on MSEL and VABS scores. *This figure shows spaghetti plots of MSEL (**A**) and VABS (**B**) subscales, whereby color indicates deciles defined by pre-treatment developmental quotients (low-to-high deciles follow blue-to-red color change). The x-axis shows age in months, while the y-axis shows age-equivalent scores in months. Individual trajectories are shown with transparent lines, while the decile group trajectories are shown overlaid with thicker lines.*

Differential rates of growth for MSEL subscales as a function of pre-treatment developmental quotient

Furthermore, MSEL age-equivalent scores showed an interaction between age and pre-treatment developmental quotient that can be described as a 'fanning-out' effect of steeper developmental trajectories for individuals higher in pre-treatment developmental quotient, but progressively less steep and much slower trajectories for individuals lower in pre-treatment developmental quotient (Figure 8A). Thus, rate of growth for MSEL age-equivalent scores markedly changes depending on an individual's starting developmental quotient before treatment begins. This 'fanning-out' effect stands in stark contrast to the lack of such an interaction effect between age and age at treatment start on MSEL subscales, whereby developmental trajectories are largely stable independent of when an individual started the intervention (Figure 7A).

Differential rates of growth for VABS Communication as a function of age at treatment start

Interactions between age and age at treatment start or pre-treatment developmental quotient were largely absent across VABS domains. An exception however, was the presence of a significant interaction between age and age at treatment start for the VABS Communication domain. Here we see that developmental trajectories are upwards slanting in slope in individuals who started treatment earlier, whereas these slopes progressively flatten as individuals start treatment later (Figure 7B). This effect potentially indicates that communication skills measured by the VABS may progressively get better over development in individuals that start treatment relatively earlier, whereas there is very little evidence for such improvement over time in communication skills in those that started treatment relatively later.

Differential rates of growth on ADOS CSS as a function of age at treatment start and pre-treatment developmental quotient

When examining autism symptom severity with ADOS CSS scores, we identified interesting interactions with age and age at treatment start or pre-treatment developmental quotient. The interaction between age and pre-treatment developmental quotient can be understood as a possible regression to the mean phenomenon, whereby individuals with the lowest pre-treatment developmental quotient start out with very high ADOS CSS scores, but which subsequently drop over development. In contrast, individuals with higher pre-treatment developmental quotients start out with lower ADOS CSS scores but then have slightly upwards trajectories that converge around the same severity levels as other individuals by 60 months of age (Figure 9A).

A very different type of interaction between age and age at treatment start exists for ADOS CSS scores. Here the interaction can be described as declining (i.e. progressive improvement over time) trajectories of ADOS CSS scores in individuals that started treatment earliest. This can be contrasted to progressive flattening or stabilization of ADOS CSS trajectories in individuals that started treatment later (Figure 9B).

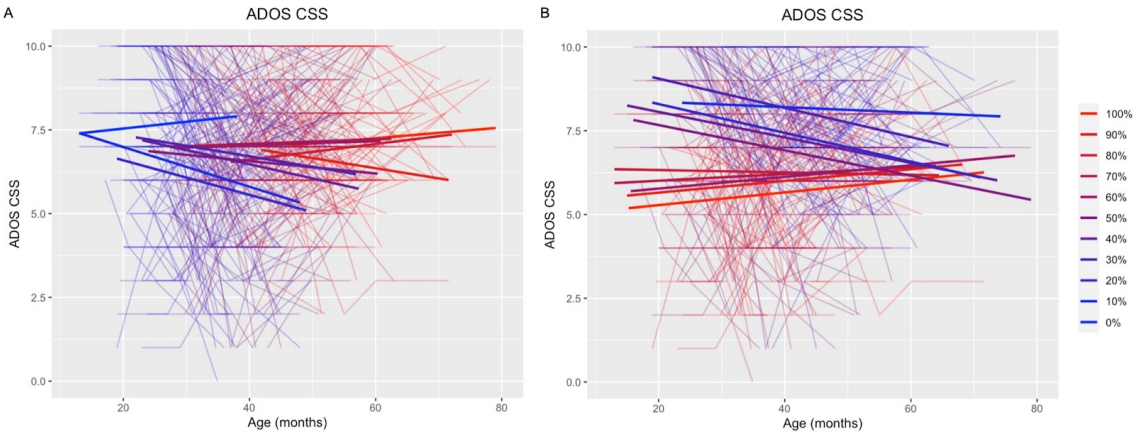

Figure 9: Effect of age at treatment start (A) and pre-treatment developmental quotient (B) on ADOS CSS scores*. This figure shows spaghetti plots of ADOS CSS scores whereby color indicates deciles defined by age at treatment start (**A**) and pre-treatment developmental quotient (**B**). The x-axis shows age in months, while the y-axis shows age-equivalent scores in months. Individual trajectories are shown with transparent lines, while the decile group trajectories are shown overlaid with thicker lines.*

3.4 Discussion

In this work we present insights from a mega-analysis of a large international consortium of autism early intervention researchers. This mega-analysis was positioned to facilitate answers to primary questions centered around autism early intervention - that is, *what works*, *for whom*, and *for what* (Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020). To target *what works* and *for what* questions, we focused on the contrast of one of the potentially more promising naturalistic developmental behavioral intervention (NDBI) in ESDM to other types of early interventions that are commonly used in community settings as the typical treatment-as-usual option. Congruent with some of the past works on ESDM (Dawson et al., 2010; Estes et al., 2015; Rogers et al., 2019; Waddington et al., 2016), we found there is some evidence that ESDM has beneficial effects on development of skills, particularly in receptive language and non-verbal (MSEL VR) cognitive skills. These effects manifest after controlling

for a variety of other factors in the model, such as variability inherent to different datasets, treatment intensity, sex, age at treatment start, pre-treatment developmental quotient. While the insights here are not couched within a large and well-controlled randomized double-blind study, they complement insights from meta-analysis (Fuller et al., 2020; Wang et al., 2022) that also suggests some small beneficial effects when compared to other types of community early interventions. A caveat to this result is that the positive effects of ESDM are relatively small and exclusive to some subscales of MSEL but were not apparent on VABS and ADOS. Furthermore, inspection of spaghetti plots reveals considerable heterogeneity in individual trajectories within ESDM. This underscores the fact that while ESDM may be on-average promoting some developmental skills better than COM/TAU, the bigger questions should be to decompose the *for whom* question and answer more precisely how ESDM may have bigger impact for some individuals (Godel et al., 2022; Lombardo, Busuoli, et al., 2021).

One of the key insights enabled by mega-analysis rather than meta-analysis or smaller scale individual studies is the ability to isolate individual factors and their key role in moderating developmental trajectories throughout the course of early intervention. Several factors tested here in this mega-analysis have been previously theorized as being potentially important individualized predictors of outcomes in the context of early intervention, particularly age at treatment start (Reichow, 2012; Vivanti et al., 2019; Warren et al., 2011), pre-treatment developmental quotient (Godel et al., 2022; Howlin et al., 2009; Rogers et al., 2019), treatment intensity (Rodgers et al., 2020a; Rogers et al., 2012), and sex (Crank et al., 2021; McVey et al., 2017). Here we find ubiquitous and strong impact for variables such as age at treatment start and pre-treatment developmental quotient across all variables examined (MSEL, VABS, ADOS). These individual baseline characteristics moderate developmental change over the course of treatment across all outcomes in both intervention groups. In contrast,

sex and treatment intensity have little to no impact. These effects are not just main effects, but also interact with age, indicating that they have differential impact on rates of growth throughout the intervention. For example, individuals high in pre-treatment developmental quotients show the highest rates of growth on MSEL subscales, while those that are relatively lower in pre-treatment developmental quotients show the most limited and slowest growth. This phenomenon is consistent with a 'rich get richer' effect and underscores that a potential important factor predicting response to treatment may be less based on type of treatment and more based on individualized characteristics of the child before treatment begins. What may underlie such effects at the level of mechanisms and biology is not well understood. However, initial studies have suggested that experience-dependent biology factors (e.g., epigenetic mechanisms such as histone acetylation) may be differentially impacted by those with variable rates of growth during an intervention (Lombardo, Busuoli, et al., 2021).

Much discussion has also centered around the push for earlier diagnosis leading to earlier intervention (Pierce et al., 2011, 2021; Zwaigenbaum et al., 2015). The theoretical idea behind this push is to capitalize on greater earlier neural plasticity to facilitate more positive outcomes for children (Dawson, 2008; Piven et al., 2017). Our findings illustrating the important impact age at treatment start has on treatment outcomes and trajectories is consistent with this theoretical idea. For example, ADOS CSS scores show declining rates of growth (e.g., lessening of symptom severity) in those that started treatment earlier, while those that started treatment relatively later show trajectories that level off and are relatively flat and stable. Similarly, VABS communication scores show slight positive slopes indicative of improving skills in those that started treatment relatively earlier, while those that started relatively later have trajectories that flatten out and stabilize. Overall, our results provide strong and definitive support for advocacy for earlier diagnosis and intervention, as age at treatment start is one of the most important moderating factors that could change individual

development during treatment. Unlike factors that are inherent to the child, which society has less control over, society does have some control over how it implements services for early diagnosis and treatment. Our results strengthen the backbone behind such advocacy and illustrate clearly the importance of starting treatment as early as possible to promote positive outcomes.

There are several caveats and limitations to underscore. First, the current mega-analysis was not restrictive in its inclusion criteria and as such, many of the contributing datasets are not well-controlled randomized double-blind studies. While such studies are a gold standard for evaluating treatment efficacy, the broader inclusion of other studies allowed for much wider reach and potentially enhanced generalizability to what is the reality in most community settings. Second, our contrast tailored to assess the what works question was limited to an assessment of how ESDM compares to a range of other diverse types of interventions commonly used in the community. As such, not enough datasets exist for a full comparison of individual treatment types within the COM/TAU grouping we used for the current analysis. Thus, while the results suggest some potential for ESDM, they do not necessarily point to the ineffectiveness of other interventions lumped into the COM/TAU group. Third, although we have tested for effects like sex, due to the still limited number of actual females (e.g., around 60 individuals per each treatment type), statistical power to robustly test for sex differences in this study may be limited.

In summary, with mega-analysis we have highlighted the prominent impact of factors like age at treatment start and pre-treatment developmental quotient as key predictors of individual differences in outcomes related to early intervention. Contrasting treatment types, there are also small effects that indicate the potential of NDBI interventions like ESDM over and above COM/TAU interventions. Other variables such as sex and treatment intensity had little effect on treatment outcomes. However, the lack of association between the intensity

and treatment outcomes should be considered with caution, since the heterogeneity present in our sample in terms of study designs, duration, dosage and delivery of interventions may have limited our analysis. This work supplements and expands insights from individual smaller-scale studies as well as on-average inferences that can be gleaned from meta-analytic work. The work also strengthens the knowledge base behind important key factors that can explain heterogeneity of outcomes during intervention. This is a key frontier for the future as we move closer towards the path of precision medicine applied to early intervention in autism.

Chapter 4: General discussion

In this final chapter, we consider implications and perspectives about research findings described in the thesis. Since an extensive discussion of each specific project has been provided in the respective chapters, here we focus more on the conclusions and limitations of these two works, and we indicate possible ways to further our research.

Chapter 2 describes our first work, where we seek to better understand how to predict differential outcomes, and which are the biological mechanisms underlying the individual differences in early intervention responses. With this aim, we used blood leukocyte gene expression patterns as possible biological predictors, and we investigated whether pre-treatment clinical behavioral and gene expression patterns could predict the developmental trajectories during early intervention. Findings confirmed that clinical behavioral characteristics at the baseline are overall predictive of outcomes, but we also found that pre-treatment blood leukocyte gene expression levels can predict treatment slopes. From this investigation there are two insights to point out: first, with longitudinal trajectories as outcome measures, we found that a simple snapshot of clinical measures at the baseline is predictive of how steep a child's slope trajectory will be during early intervention. Second, the new revelation is that also biological characteristics, such as pre-treatment blood leukocyte gene expression patterns, might be informative of individual treatment trajectories. Digging deeper into gene expression characteristics, we also found that the treatment relevant genes, prioritized by our LASSO model, are enriched for genes that are differentially histone acetylated in post-mortem cortical tissue of autistic patients. These findings may reveal that epigenetic mechanisms such as histone acetylation may be a key biological process which could explain why children's responses to early intervention widely differ. This idea, although it needs to be confirmed by future

works, fits well with general ideas regarding histone acetylation as one of the epigenetic processes regulating activity-dependent gene expression, which could influence the experience-dependent learning and memory processes. Thus, it might be that children who have differences in histone acetylation mechanisms are more or less susceptible to experience-dependent regulation of gene expression, and they may benefit differently from environmental changes such as early intervention. The last take-home message from this work is the importance of starting intervention early, ideally before 24 months. We found that toddlers who started treatment before 24 months showed greater gains on average, and reduced variability of treatment slopes, than those who started treatment after 24 months. This result highlights why early intervention is essential for treatment gains and for the prediction of treatment trajectories, that is much more difficult when the child begins treatment later.

Chapter 3 describes our second empirical work, where we addressed primary early intervention questions. We provided information on what works and for what questions, but most importantly, we decomposed the for whom question. For this purpose, we employed a mega-analysis approach combining raw data from multiple datasets into a larger, single sample. This allowed us to isolate individual factors and retain more detailed information than a meta-analysis of summary statistics. Thanks to the AEIR consortium, we were able to report results of a mega-analysis which pooled together 645 autistic children across two treatment types, Early Start Denver Model (ESDM) or other treatment as usual/community interventions (TAU/COM). Furthermore, unlike most recent large-scale studies of early intervention research (Fuller et al., 2020; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020), here we used longitudinal data that enabled us to describe the individual variability over time of most common treatment outcomes, such as autism symptom severity, adaptive behavior and cognitive domains. Also, it should be noted that the same tool to measure each specific outcome domain was used across studies, increasing the

comparability among participants and the validity of our assumptions. This is an additional strength of our work, since different methodological and measurement choices represent a source of inconsistency present in previous meta-analytic works (Crank et al., 2021; Rodgers et al., 2021). To answer what works and for what questions we compared ESDM to COM/TAU, and we found some evidence that ESDM has beneficial effects on receptive language and non-verbal cognitive skills. Although the result reported here is not supported by RCT studies, it complements findings of a recent meta-analysis (Fuller et al., 2020) which also reported small positive effects of ESDM in language and cognitive areas. Given the heterogeneous and relatively subtle treatment type effects at the group level, we then focused on individual factors and their role in predicting outcomes throughout the course of early intervention. Our findings revealed robust evidence that age at treatment start and cognitive level at the baseline have a strong and ubiquitous impact, in both intervention types, on all variables examined. Additionally, these individual characteristics do not just have main effects, but also interact with age, revealing that they have differential impact on developmental trajectories during intervention. For example, cognitive level at intake predicted cognitive trajectories, consistently with a 'rich get richer' effect, where steeper developmental trajectories are observed for children who started higher in cognitive skills. Age at treatment start also predicted cognitive scores on average, but showed a stable effect on trajectories, meaning that in this case, age at intake did not predict how fast a child acquired cognitive skills. As suggested by previous authors (Chen et al., 2022), here we confirmed the importance of assessing individual developmental trajectories, since including the effect of age as a moderator may depict a different picture of predictors. Another important finding of our mega-analysis is the positive influence of starting intervention early on all outcomes, and also in the trajectories of autism symptom severity. We observed a lessening of symptom severity in children that started treatment earlier, while those that started treatment later showed flat or stable trajectories. Therefore, consistently with our first investigation, empirical findings of this

research strongly support the theoretical idea behind the value of earlier intervention.

4.1 Limitations & Future Directions

Specific limitations of each empirical work are discussed within each chapter. However, there are some general limitations that need to be addressed to interpret the results reported in this thesis. A primary observation to consider is about the novel finding from the work described in Chapter 1, in which blood leukocytes gene expression patterns were found to be informative for predicting developmental change. Although the sample size of this study is relatively moderate for what is typical in most gene expression and treatment studies, the result identified need to be confirmed in future works with larger samples, to meet basic criteria for validity and replicability. Our finding should be considered as a starting point for future trials that aim to provide evidence that different biological mechanisms do relate to differential outcomes related to treatment.

A limitation, shared by the two works described, is that we did not adopt well-controlled randomized double-blind designs to support our inferences about treatment effects. The first study is correlational in nature and does not include a control group to compare the effect of the early intervention program examined. Therefore, the interpretation of treatment-related learning cannot be dissociated from possible maturational effects. In the second work, to provide a more representative picture of real-world application of early intervention, we included a large number of studies with a wide range of study designs, which comprised both RCTs and less constrained interventions in community settings. However, without filtering or controlling for study quality/bias, our evidence of positive effects of ESDM is limited. Given the current scarcity of meta- or mega-analyses of RCT studies on ESDM, it seems reasonable to suggest that more RCT studies

with large sample sizes are needed, to elucidate whether at the group level ESDM intervention is a more optimal choice compared to other interventions, (Crank et al., 2021; Sandbank, Bottema-Beutel, Crowley, Cassidy, Dunham, et al., 2020; Wang et al., 2022) and for which individuals ESDM works best.

A caveat of our mega-analysis relates to the small exclusive effects of ESDM on receptive language and non-verbal cognitive skills, while no positive effects were evident on adaptive behavior and autism symptom severity outcomes. This may indicate that the ESDM intervention is less effective at targeting these domains. However, it should be noted that the duration of the intervention ranged from 3 to 27 months, and adaptive behavior outcomes, measured by VABS scores, are considered more related to long-term goals since they are strongly associated with later life outcomes (e.g., later independent living, educational attainment) (De Bildt et al., 2005; Farley et al., 2009). Thus, it is possible that adaptive skills learned in the intervention environment need a longer period of practice before they can be integrated as appropriate adaptive behaviors in the natural home environment (Ben-Itzchak et al., 2014). For this reason, it might be important to examine outcomes during long-term follow-up. Also, in the case of autism symptom severity outcome, the use of ADOS might lead to some measurement issues, since it was designed to capture relatively stable features of autism symptomology and was not created with the intention of measuring treatment-related changes (Fuller et al., 2020; Klintwall et al., 2015). Generally, all outcome measures present in the mega-analysis, are not considered sensitive or valid measures of short-term treatment-related changes, as they were originally intended to measure stable traits. Although these measures are the most commonly reported, and gains in these measures suggest solid changes in children's behaviors, they are not considered ideal for outcome research (Klintwall et al., 2015). Future treatment studies should employ measures designed to specifically assess treatment response, such as Brief Observation of Social

Communication Change (BOSCC), a more recent measure created for capturing treatment response for social communication behaviors (Grzadzinski et al., 2016).

The general aim of this research was to get closer to precision medicine goals, going beyond on-average effects found in previous studies, and disentangling individual variability in treatment related outcomes. With this purpose, we found that individual baseline characteristics such as blood gene expression patterns, clinical characteristics and the child's age are highly relevant for predicting outcomes and trajectories of treatment. Further directions of these works should focus on creating more specific intervention subgroups using participants characteristics. Given the relevance of the cognitive level at the baseline, we expect that, within a small age range, this factor could be used as stratifier to identify specific subgroups of autistic children who respond better to early interventions than others. This might give us a better understanding of why early interventions, such as ESDM, work better for some but not for all individuals. As done in previous studies (Ben-Itzchak et al., 2014), to subgroup autistic population into cognitive ability subgroups, we can use standard deviation cutoff scores based on developmental quotient at the baseline. Alternatively, unsupervised data-driven clustering approaches can be applied. In our lab was developed reval algorithm (Landi et al., 2021) and used to identify consistent subtypes in autism (Mandelli et al., 2023). This model could be used to stratify autism population into subgroups based on cognitive level at intake, and then apply it to split ESDM and community treatment participants into subtypes. Subtypes identified from this method can be then analyzed for differences in treatment response (ESDM vs Community). If subtypes can be identified with this unsupervised data-driven clustering approach, this model can be applied in the future to gain a better understanding of how individuals may respond to other early interventions.

To conclude, this research highlights the importance of assessing individual baseline characteristics to explain the heterogeneity of early intervention outcomes. Age of treatment start, initial cognitive level and early intervention program, such as ESDM, may work as protective factors to promote the development of autistic children. Among these factors, services for early diagnosis and treatment can be controlled and improved by society to some extent, while the set of cognitive abilities with which a child is born are out of control. However, experience-dependent learning processes are shaped by the environment (Gräff & Tsai, 2013). For this reason, effective interventions are not expected to "cure" autism, but to find a balance between accommodation of autistic features and the mitigation of harmful behaviors to focus on core developmental skills, which are relevant for later learning and reaching independence (Leadbitter et al., 2021; Rodgers et al., 2021).

Hopefully, this work will be useful in further advancing research on autism early intervention, providing insights to address questions that remain unanswered.

Tables

Table 1. Pre-treatment clinical characteristics. *Abbreviations: ADOS, autism diagnostic interview schedule; MSEL, Mullen Scales of Early Learning; VABS, Vineland Adaptive Behavior Scales; SC, social-communication; RRB, restrictive repetitive behaviors; EL, expressive language; RL, receptive language; VR, visual reception; FM, fine motor; ELC, early learning composite; SD, standard deviation*

	Male	**Female**
Sex	33	8
	Mean	**SD**
Age at Treatment Intake	22.78	4.03
ADOS Total	14.27	6.02
ADOS SC	12.17	5.41
ADOS RRB	6.17	2.11
MSEL EL	31.39	10.62
MSEL RL	29.10	11.22
MSEL VR	41.29	9.65
MSEL FM	41.63	11.38
MSEL ELC	73.83	15.31
VABS Communication	77.12	14.38
VABS Socialization	85.51	12.52
VABS Daily Living Skills	89.05	11.99
VABS Motor	94.83	11.98

VABS Adaptive Behavior Composite	84.27	12.34

Table 2: Gene Enrichment Analysis. *This table shows results of gene set enrichment analysis for treatment-relevant genes. Abbreviations: GO, gene ontology.*

Biological Process (GO)			
GO-term	**description**	**count in network**	**false discovery rate**
GO:0032880	regulation of protein localization	29 of 901	0.0379
GO:0016192	vesicle-mediated transport	48 of 1699	0.0043

Cellular Component (GO)			
GO-term	**description**	**count in network**	**false discovery rate**
GO:0005829	cytosol	101 of 4958	0.0019
GO:0070013	intracellular organelle lumen	98 of 5162	0.0190
GO:0005737	cytoplasm	186 of 11238	0.0037

UniProt Keywords			
keyword	**description**	**count in network**	**false discovery rate**
KW-0007	Acetylation	74 of 3335	0.0024

Table 3: Enrichments with autism-relevant gene lists. *This table shows results of enrichment analysis for treatment-relevant genes and autism-relevant gene lists. Abbreviations: DE, differentially expressed; DA, differentially histone acetylated; OR, enrichment odds ratio; FDR, false discovery rate.*

Gene List	**Odds Ratio (OR)**	**p-value**	**false discovery rate (FDR)**
SFARI ASD	0.73	0.85	0.86
ASD DE Downregulated	0.68	0.86	0.86
ASD DE Upregulated	1.46	0.13	0.23

ASD DA Prefrontal Cortex Upregulated	1.64	0.007	0.04
ASD DA Prefrontal Cortex Downregulated	1.34	0.09	0.21
ASD DA Temporal Cortex Upregulated	1.24	0.16	0.23
ASD DA Temporal Cortex Downregulated	1.51	0.01	0.04

Table 4: Descriptive statistics of baseline characteristics between treatment types. *Abbreviations: ESDM, Early Start Denver Model; COM/TAU, Community/Treatment-As-Usual; Pre-treatment DQ, Pre-treatment Developmental Quotient; M, Mean; SD, Standard Deviation.*

	ESDM	**COM/TAU**
Sample size	304	341
Male	239	278
Female	65	63
Age at intake: M (SD)	30.46 (8.69)	33.61 (10.21)
Pre-treatment DQ: M (SD)	62.25 (16.94)	61.92 (19.15)
Intensity: M (SD)	15.60 (12.02)	10.21 (11.13)

Table 5: Longitudinal analysis for ADOS, VABS and MSEL scores. *Linear Mixed Effect models were utilized for each subscale of MSEL, for each domain of VABS and one model for ADOS. ADOS calibrated severity scores (CSS)* ***(A)****, MSEL age-equivalent scores* ***(B, C, D, E)*** *and VABS standardized scores* ***(F, G, H, I)*** *were the dependent variables. Fixed effects for all models were age, treatment type, treatment intensity, sex, age at treatment start, and pre-treatment developmental quotient. Interaction effects between these variables were also modeled as fixed effects. Random effects were utilized to account for subject-specific age intercepts and slopes and to capture the nested within-study factors.*

A

ADOS CSS	F	p-value	fdr
Age	29.16	1.22 e-07	7.35 e-07
Treatment type	0.68	4.10 e-01	5.67 e-01
Sex	0.72	3.96 e-01	5.68 e-01
Age at start	12.87	3.58 e-04	1.43 e-03
Pre-treatment DQ	113.76	4.16 e-24	4.99 e-23
Intensity	0.08	7.80 e-01	7.96 e-01
Treatment * age	0.39	5.32 e-01	6.39 e-01
Treatment * sex	0.635	4.26 e-01	5.68 e-01
Age at start * age	8.05	5.04 e-03	1.01 e-02
Pre-treatment DQ * age	10.25	1.55 e-03	4.63 e-03

Table 5

B

MSEL		F	p-value	fdr
Expressive Language	**Age**	1503.35	3.18 e-161	3.82 e-160
	Treatment type	1.63	2.03 e-01	2.70 e-01
	Sex	0.72	3.96 e-01	4.18 e-01
	Age at start	54.74	1.86 e-12	5. 61 e-12
	Pre-treatment DQ	516.22	5.22 e-70	3.14 e-69
	Intensity	2.90	1.03 e-01	1.76 e-01
	Treatment * age	2.44	1.18 e-01	1.77 e-01
	Treatment * sex	0.65	4.18 e-01	4.18 e-01
	Age at start * age	3.06	8.08 e-02	1.62 e-01
	Pre-treatment DQ * age	105.85	1.79 e-22	7.17 e-22

C

MSEL		F	p-value	fdr
Receptive Language	**Age**	1564.33	3.21 e-167	3.85 e-166
	Treatment type	6.60	2.22 e-02	4.45 e-02
	Sex	2.53	1.12 e-01	1.68 e-01
	Age at start	80.83	6.24 e-11	1.87 e-10
	Pre-treatment DQ	507.77	2.64 e-68	1.58 e-67
	Intensity	0.49	5.77 e-01	6.88 e-01
	Treatment * age	8.49	3.69 e-03	8.87 e-03
	Treatment * sex	0.16	6.88 e-01	6.88 e-01
	Age at start * age	1.05	3.06 e-01	4.08 e-01
	Pre-treatment DQ * age	72.21	2.99 e-16	1.19 e-15

D

MSEL		F	p-value	fdr
Visual Reception	**Age**	1.39 e+03	1.13 e-158	1.36 e-157
	Treatment type	3.08 e-01	5.79 e-01	6.96 e-01
	Sex	1.26	2.62 e-01	3.93 e-01
	Age at start	4.60 e+01	5.57 e-11	1.67 e-10
	Pre-treatment DQ	4.46 e+02	4.13 e-63	2.48 e-62
	Intensity	5.48	2.84 e-02	5.68 e-02
	Treatment * age	5.33	2.12 e-02	5.09 e-02
	Treatment * sex	2.84 e-05	9.95 e-01	9.957464e-01
	Age at start * age	2.16 e-02	8.83 e-01	9.63 e-01
	Pre-treatment DQ * age	6.69 e+01	2.76 e-15	1.11 e-14

E

MSEL		F	p-value	fdr
Fine Motor	**Age**	1.38 e+03	6.86 e-151	8.23 e-150
	Treatment type	2.56 e-01	6.13 e-01	7.36 e-01
	Sex	1.60 e-01	6.89 e-01	7.52 e-01
	Age at start	1.89 e+01	1.94 e-05	5.83 e-05
	Pre-treatment DQ	3.22 e+02	7.66 e-51	4.59 e-50
	Intensity	2.95 e-01	5.95 e-01	7.36 e-01
	Treatment * age	4.28	3.90 e-02	7.82 e-02
	Treatment * sex	1.17 e-03	9.73 e-01	9.73 e-01
	Age at start * age	2.19	1.38 e-01	2.38 e-01
	Pre-treatment DQ * age	7.70 e+01	4.96 e-17	1.98 e-16

F

VABS		F	p-value	fdr
Socialization	**Age**	6.03	1.44 e-02	5.79 e-02
	Treatment type	0.04	8.35 e-01	9.74 e-01
	Sex	0.02	8.79 e-01	9.74 e-01
	Age at start	16.98	4.37 e-05	2.62 e-04
	Pre-treatment DQ	148.24	1.61 e-29	1.94 e-28
	Intensity	0.88	3.53 e-01	7.06 e-01
	Treatment * age	1.83	1.76 e-01	4.23 e-01
	Treatment * sex	0.01	9.04 e-01	9.74 e-01
	Age at start * age	2.50	1.147 e-01	3.44 e-01
	Pre-treatment DQ * age	0.27	6.05 e-01	9.07 e-01

G

VABS		F	p-value	fdr
Daily Living	**Age**	1.42	2.33 e-01	4.66 e-01
	Treatment type	1.46	2.29 e-01	4.66 e-01
	Sex	4.77 e-01	4.90 e-01	6.62 e-01
	Age at start	5.55	1.89 e-02	7.58 e-02
	Pre-treatment DQ	2.00 e+02	1.09 e-37	1.31 e-36
	Intensity	4.37 e-03	9.48 e-01	9.48 e-01
	Treatment * age	1.83	1.76 e-01	4.23 e-01
	Treatment * sex	1.11	2.93 e-01	5.01 e-01
	Age at start * age	4.62 e-01	4.97 e-01	6.63 e-01
	Pre-treatment DQ * age	8.04 e-03	9.29 e-01	9.48 e-01

H

VABS		F	p-value	fdr
Communication	**Age**	1.25 e+02	3.02 e-26	1.81 e-25
	Treatment type	1.25	2.65 e-01	3.97 e-01
	Sex	1.05	3.05 e-01	4.07 e-01
	Age at start	5.18 e+01	1.85 e-12	7.41 e-12
	Pre-treatment DQ	3.25 e+02	8.91 e-55	1.07 e-53
	Intensity	3.36	6.96 e-02	1.67 e-01
	Treatment * age	7.82 e-03	9.29 e-01	9.29 e-01
	Treatment * sex	7.29 e-02	7.87 e-01	8.58 e-01
	Age at start * age	6.27	1.28 e-02	3.86 e-02
	Pre-treatment DQ * age	1.83 e-01	6.69 e-01	8.03 e-01

I

VABS		F	p-value	fdr
Motor	**Age**	1.09 e+01	1.01 e-03	6.07 e-03
	Treatment type	5.96 e-02	8.08 e-01	8.81 e-01
	Sex	3.56	5.99 e-02	1.44 e-01
	Age at start	1.36	2.44 e-01	3.66 e-01
	Pre-treatment DQ	8.10 e+01	8.27 e-18	9.92 e-17
	Intensity	3.53	8.14 e-02	1.63 e-01
	Treatment * age	2.13 e-01	6.44 e-01	7.73 e-01
	Treatment * sex	1.95	1.63 e-01	2.79 e-01
	Age at start * age	4.00	4.65 e-02	1.39 e-01
	Pre-treatment DQ * age	7.21	7.79 e-03	3.12 e-02

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