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RESEARCH ARTICLE

Rare variants in the outcome of social skills group training for autism

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Abstract

Exome sequencing has been proposed as the first-tier genetic testing in autism spectrum disorder (ASD). Here, we performed exome sequencing in autistic individuals with average to high intellectual abilities ($N = 207$) to identify molecular diagnoses and genetic modifiers of intervention outcomes of social skills group training (SSGT) or standard care. We prioritized variants of clinical significance (VCS), variants of uncertain significance (VUS) and generated a pilot scheme to calculate genetic scores of rare and common variants in ASD-related gene pathways. Mixed linear models were used to test the association between the carrier status of VCS/VUS or the genetic scores with intervention outcomes measured by the social responsiveness scale. Additionally, we combined behavioral and genetic features using a machine learning (ML) model to predict the individual response. We showed a rate of 4.4% and 11.3% of VCS and VUS in the cohort, respectively. Individuals with VCS or VUS had improved significantly less after standard care than non-carriers at post-intervention ($\beta = 9.35$; $p = 0.036$), while no such association was observed for SSGT ($\beta = -2.50$; $p = 0.65$). Higher rare variant genetic scores for synaptic transmission and regulation of transcription from RNA polymerase II were separately associated with less beneficial ($\beta = 8.30$, $p = 0.0044$) or more beneficial ($\beta = -6.79$, $p = 0.014$) effects after SSGT compared with standard care at follow-up, respectively. Our ML model showed the importance of rare variants for outcome prediction. Further studies are needed to understand genetic predisposition to intervention outcomes in ASD.

Lay Summary

Social skills group training is one of the most common behavior interventions aiming at autistic children and adolescents, but the outcome varies between

Sven Bölte and Kristiina Tammimies contributed equally to this study.

individuals. In this study, we showed that rare variants identified by exome sequencing were associated with intervention outcomes. The results may guide future studies on underlying biological mechanisms and help shape more precise intervention plans for autistic individuals.

KEYWORDS
autism spectrum disorder, exome sequencing, genetic testing, rare variants, social skills group training

INTRODUCTION

The molecular genetic understanding of autism spectrum disorder (ASD) has undergone breakthroughs in research settings over the last decade, and these findings are continuously translated into clinical use (Finucane et al., 2021; Havdahl et al., 2021). Currently, the main implication of clinical genetic testing for an individual with ASD is to potentially identify a molecular diagnosis that can be used as a diagnosis specifier according to the Diagnostic & Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013). The standard recommended genetic testing for individuals with ASD has been targeted testing for Fragile X syndrome and chromosomal microarray to detect copy number variations (CNVs) (Miller et al., 2010). However, a recent consensus statement suggested that exome sequencing detecting rare genetic variants should replace chromosomal microarray as the first-tier genetic testing for neurodevelopmental disorders (NDDs), including ASD (Srivastava et al., 2019). Despite these recommendations, the utilization of genetic testing in ASD is still low (Moreno-De-Luca et al., 2020), and the usefulness of genetic information beyond a molecularly defined diagnosis is still limited.

With hundreds of genes influenced by rare variants involved in ASD, genetic findings have revealed important insights into the biology of the condition by suggesting the convergence of gene networks such as synaptic transmission and gene regulation pathways (De Rubeis et al., 2014; Ruzzo et al., 2019; Satterstrom et al., 2020). However, little knowledge is known on how the rare variants or networks affect the interventions for ASD.

In addition to rare genetic variants, polygenic risk score (PRS), which aggregates the risk of common variants from genome-wide association studies (GWAS), is associated with ASD and shows an additive effect with rare variants (Grove et al., 2019; Weiner et al., 2017). A justified assumption is that genetic information can help develop more individualized and effective interventions (Johannessen et al., 2017). Nevertheless, few studies are focused on investigating how genetic information or genetically defined subgroups of autistic individuals can be used to guide the choice or development of treatments and interventions for ASD (Berry-Kravis et al., 2016).

Machine learning (ML) prediction models are increasingly being used in relation to ASD, and studies have successfully used genetic information to predict additional genes, diagnosis, and variants pathogenicity (Asif et al., 2020; Chen et al., 2018; Krishnan et al., 2016). However, a few studies have used ML methods to predict intervention outcomes in ASD and other mental disorders by integrating genetic and clinical predictors (Kautzky et al., 2015; Lenhard et al., 2018).

As social communication and interaction challenges are the core features of ASD, the most common interventions offered to autistic individuals target social skills or development (Chancel et al., 2020). Social skills group training (SSGT) is a behavioral intervention widely applied in ASD to ameliorate social communication difficulties, with varied responses depending on factors such as age and sex (Choque Olsson et al., 2017). In addition, our group demonstrated that both rare CNVs and common variant PRS could affect individual intervention outcomes in a randomized controlled trial evaluating the SSGT KONTAKT® (Li et al., 2020; Tammimies et al., 2019). Seeking to examine further the role of rare variants on individual outcomes of SSGT and standard care, we performed exome sequencing in the participants and leveraged all level genetic data and clinical information from the KONTAKT® trial (Choque Olsson et al., 2017), as summarized in Figure 1. Additionally, we tested the contribution of common and rare variants in ASD-related specific gene sets measured by constructed genetic scores to gain biological insights into the outcome variability. Lastly, we built a machine learning model combining clinical and genetic features to predict intervention responses within the KONTAKT® randomized controlled trial.

METHODS

Participants

All participants in this study were a part of the SSGT KONTAKT® randomized controlled trial (clinical trial identifier: NCT01854346) (Choque Olsson et al., 2017). The inclusion and exclusion criteria, participant characteristics, and the trial outcome have been reported in detail earlier (Choque Olsson et al., 2017). In brief, all the participants had a confirmed ASD diagnosis, had an IQ > 70, and at least one comorbid neurodevelopmental or psychiatric diagnosis (such as attention deficit hyperactivity disorder [ADHD], anxiety disorder, or depression).

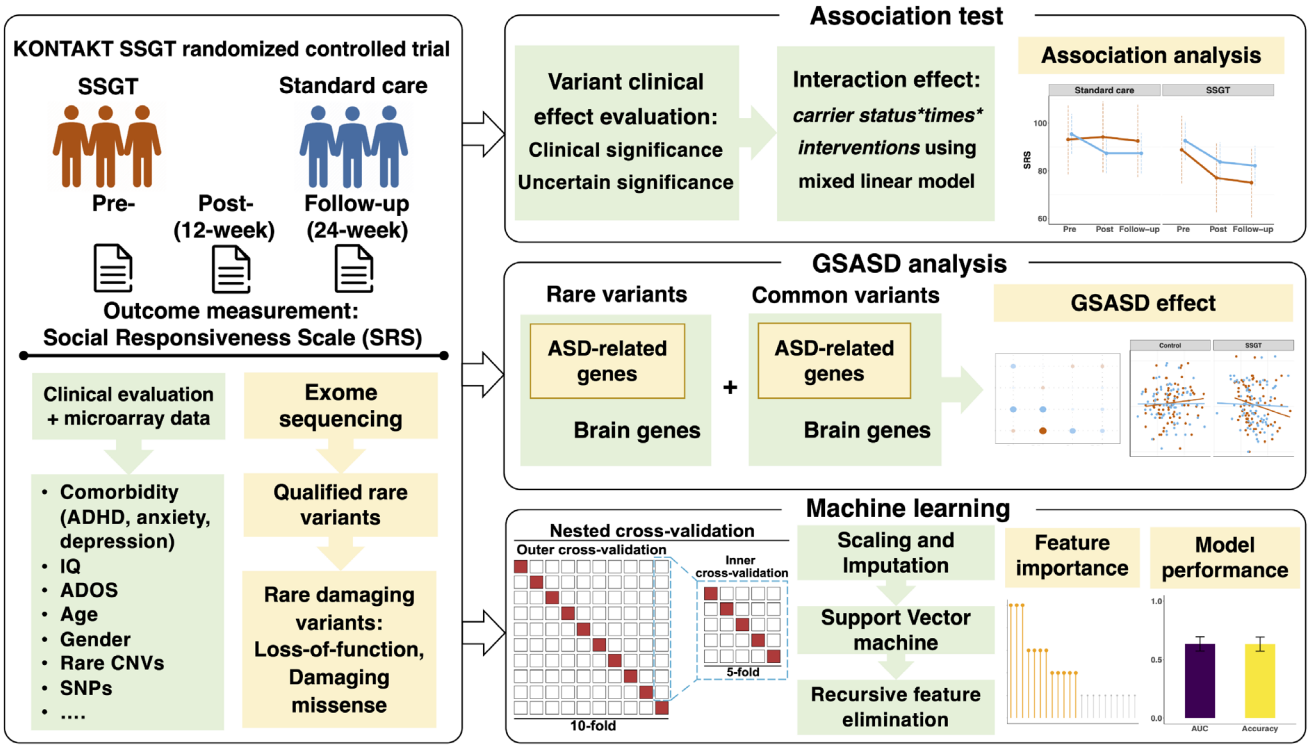

FIGURE 1 Overview of the study and performed analysis. SSGT: Social skills group training; ADHD, Attention deficit hyperactivity disorder; ADOS, Autism diagnostic observation schedule; CNV, Copy number variation; GSASD, Genetic score for ASD-related gene sets; SNP, Single nucleotide polymorphism

In KONTAKT®, participants are trained in groups of children (7–13 years) or adolescents (13–17 years). In the trial, participants were randomly assigned to either the standard care receiving any usually provided clinical intervention, or the SSGT group receiving standard care plus SSGT. A total of 207 (SSGT group: 105, standard care group: 102) participants were included in this study, who had consented to the genetic part and had available saliva samples as well as recorded parent-reported Social Responsiveness Scale (SRS) total scores (primary outcome) at baseline and either at post-intervention ($n = 186$) or at 12-week follow-up after post-intervention ($n = 169$) (Constantino & Gruber, 2005). The outcome time was controlled, and each outcome measure was recorded within two weeks at each time point (pre-, post-intervention, and follow-up).

The four subscales of the SRS probing different social behavioral traits (social awareness, social cognition, social motivation, social communication) were also used in this study as targeted outcomes (Constantino & Gruber, 2005) to explore whether specific social domains could be underlying the association with genetic factors. It is to note that the psychometric properties are not well defined for these subscales, and they were not used individually in the clinical trial as outcomes. As a part of the analysis presented in this study, we determined a binary classification outcome based on the change in the SRS from pre- to post-treatment. Participants with a > 10% decrease in the total SRS score were classified as "response to treatment". A higher value of SRS total score and subscales indicate lower social ability in certain aspects. Additional information on the other clinical measures can be found in Data S1.

Written informed consent was obtained from parents or legal guardians, and verbal consent from all participants. All the protocols and methods were followed in accordance with the Declaration of Helsinki and were approved by the ethical review board in Stockholm (Dnr 2012/385–31/4) and the clinical authorities of the two involved counties.

Variant calling and prioritization

Exome sequencing was done on NovaSeq 6000 using paired-end 2 × 150 readout. Detailed information on library preparation and quality control can be found in Data S1. After sequencing, data quality control, reads alignment, variant calling, and annotations were completed using Mutation Identification Pipeline (MIP) (Stranneheim et al., 2014) (https://github.com/Clinical-Genomics/MIP). The average sequencing coverage of coding exons within OMIM genes in all subjects was between 77.9% and 98.2% at 10X sequence depth (Figure S1). In addition, 89.9% and 88.5% of the whole exome were covered with at least 10X and 20X sequence depth, respectively.

The called variants were first filtered based on quality and minor allele frequency (Data S1). The variant effect, defined by the Sequence Ontology (http://www.sequenceontology.org/), was annotated by Ensembl Variant Effect Predictor (VEP) v92. After quality control, qualified rare variants with the effect of loss-of-function (LOF, splice donor/acceptor, frameshift, start lost, and stop gained) or damaging missense were prioritized as rare deleterious variants for clinical significance assessment. Rare deleterious variants located in developmental disorder-related genes (Table S1, see Supplemental Information), and according to the gene inheritance pattern, were considered developmental disorder-related rare variants. Furthermore, evaluation to categorize the developmental disorder-related rare variants to pathogenic/likely pathogenic, uncertain clinical effect, or benign/likely benign based on ACMG guidelines was done (Richards et al., 2015). Both pathogenic and likely pathogenic variants were classified as variants of clinical significance (VCSs). All VCSs and variants of uncertain significance (VUSs) were validated by Sanger sequencing.

Genetic scores for ASD-related gene sets (GSASD)

To test a pilot scheme for analyzing gene set specific genetic load, we constructed genetic scores for synaptic transmission (SyT) genes (GSSyT) (GO:0007268), regulation of transcription from RNA polymerase II promoter (Pol II) genes (GSPol) (GO:0006357), curated developmental disorder-related gene list (Data S1), and genes from Simons Foundation Autism Research Initiative (SFARI, category 1, 2, and syndromic). To calculate GSASD based on the rare genetic variants, we used the number of genes with rare deleterious exome variants or rare CNVs in the ASD-related gene sets and brain gene set (Tammimies et al., 2019) (detailed information, see Data S1). The number of ASD-related genes captured by these rare CNVs and deleterious exome variants was added and divided by the number of brain-expressed genes with rare variants in each individual. Next, we generated GSASD for the common variants acquired from the previous study (Li et al., 2020). The calculation of common variants GSASD was based on set-based polygenic risk score (PRS) using a new feature of PRSice named PRSet (https://www.prsice.info/quick_start_prset/). We chose the largest ASD GWAS of Grove et al. (Grove et al., 2019) as reference data to calculate set-based PRS for ASD. The ratio between ASD-related sets and brain PRSs was represented as GSASD for common variants.

Statistical analyses

We applied a mixed linear model to assess whether VCS/VUS carrier status was associated with intervention outcome measured by parent-reported SRS total score and four subscales. We included a three-way interaction: carrier status*time points*interventions together with lower-order interactions, age, sex, PRS for ADHD (P-threshold 1.0), clinically significant CNVs as fixed factors, and the clinics in which the individuals had participated in the intervention and individual IDs as random factors in the model. In addition, to measure the separate effect of VCS/VUS on SSGT or standard care, the two-way interactions of carrier status*time points were tested using the same mixed linear model in SSGT and standard care subgroups separately. Furthermore, we also performed linear regression as a secondary model to test whether VCS/VUS and ASD PRS (P-threshold 0.5) independently affect the intervention outcomes.

Similarly, we computed a mixed linear model to investigate the effect of GSASD on the intervention outcomes. We combined two three-way interactions of $GSASD_r$*time points*interventions and $GSASD_c$*time points*interventions with all lower-order interactions, age, sex as fixed factors, and the same random factors as earlier. Furthermore, linear regression was performed to test the interaction of $GSASD_c$ and $GSASD_r$ using the change in SRS total score as the outcome with sex and age as cofactors. p-values were corrected after multiple testing (Benjamini & Hochberg, 1995).

Machine learning prediction

We used a binary intervention outcome calculated from the change in the SRS raw score for the machine learning prediction models. The responders were indicated as >10% decrease in the SRS total score or 10-point decrease as an alternative model (see Data S1). The participants that did not reach these changes were coded as non-responders. The prediction models were constructed using the participant characteristics, comorbidity diagnoses, treatments, autism-related scales assessed at pre-treatment, and genetic information of rare and common variants. Detailed feature information is listed in Table S2. We performed nested cross-validation by splitting total data into training and validation sets using outer cycle (10-fold) and then splitting the training set again into training and test data using inner cycle (5-fold). A linear support vector machine (SVM) was employed for prediction, and recursive feature elimination was implemented to rank the most important features. We used the area under the receiver operating characteristic curve (AUC) as model performance measurement. Other metrics, such as sensitivity, specificity, and accuracy, were evaluated at each outer cycle cross-validation. More detailed information on the alternative models can be found in Data S1.

RESULTS

Clinically significant variants and phenotype characteristics

Exome sequencing data were analyzed for 207 individuals from the SSGT KONTAKT® randomized controlled trial. Three individuals were excluded due to the low sample quality. In the remaining data from 204 individuals, 102 (50.0%) were from the SSGT group, 60 (29.4%) were female, and 124 (60.8%) were adolescents. Based on our variant prioritization scheme, each individual had on average 54 rare deleterious variants, of which four were putatively developmental disorder-related rare variants (Figure S2). From these, we identified nine variants of clinical significance (VCS) in nine carriers (4.4%) and 24 variants of uncertain significance (VUS) in 23 carriers (11.3%) following the ACMG guideline (Richards et al., 2015) (Table 1, Table S3). All VCS (five indels, four single nucleotide variants [SNVs]) were rare loss-of-function variants in autosomal dominant genes (Table 1). In total, 29 individuals had either one VCS or VUS, and two individuals carried two different variants (one individual with a VUS and a VCS, and another individual with two VUSs), respectively. There were no significant differences in parental reported total Social Responsiveness Scale (SRS) score at pre-treatment ($p = 0.68$), IQ ($p = 0.86$), or Autism Diagnostic Observation Schedule (ADOS) social communication total scores ($p = 0.28$) between individuals with VCS/VUS and those without these variants (Figure S3). We also tested the difference after removing individuals with earlier reported clinically significant CNVs (Tammimies et al., 2019), and found similar non-significant results (pre-treatment SRS: $p = 0.69$, IQ: $p = 0.82$, ADOS: $p = 0.30$).

In addition to exome sequencing, we used our earlier acquired data on clinically significant rare CNVs and common variants polygenic risk score (PRS) for ASD (Li et al., 2020; Tammimies et al., 2019). The distribution of PRS for ASD did not differ between individuals with or without VCS/VUS from exome sequencing ($p = 0.37$, Figure S4a). Two individuals were carriers of both clinically significant rare CNVs and VUSs, but no individual had both clinically significant rare CNVs and VCSs ($p = 0.65$, Figure S4b). Detailed distribution of PRS for ASD and carriers with different prioritized variants were shown in Figure S4c.

Association between carrier status and SRS score

To investigate the role of VCS/VUS identified in this study on the SSGT and standard care intervention outcomes, we used parent-reported autistic traits total score on the SRS as the primary outcome and tested interactions of carrier status of prioritized variants, time points, and interventions using a mixed linear model in the whole cohort. We first analyzed individuals with VCS or VUS together in comparison with non-carriers. The interaction of carrier status and time points indicated that individuals who were carriers tended to improve less at post-intervention ($\beta = 9.22$, $p = 0.057$, Table 2, Figure 2). Further exploratory analysis of the changes in the four SRS subscales (social communication, social awareness, social cognition, social motivation) showed that the total SRS score differences were driven by changes in the social cognition subscale ([VCS + VUS]*post-intervention: $\beta = 3.34$, $p = 0.006$, Figure S5). The other three subscales did not show significant associations with the carrier status (Figure S5). A similar trend was observed when comparing carriers of VCS to non-carriers. We found VCS carriers had significantly less improvements at post-intervention ($\beta = 14.84$, $p = 0.029$, Table 2), especially for the changes of social cognition subscale (VCS*post-intervention: $\beta = 4.38$, $p = 0.0095$). No outcome difference was detected between VUS carriers and non-carriers (VUS*post-intervention: $\beta = 4.36$, $p = 0.49$, Table 2). We did not find any significant association at follow-up (Table 2). When contrasting the two intervention groups in three-way interaction, no significant association was observed with SRS total scores or any of the four subscales (Figure 2, Table 2, Figure S5). Next, to distinguish the influence of the VCS/VUS within each intervention group, we performed the association analysis for the intervention groups separately. When combining VCS and VUS carriers into a single carrier group, a significantly inferior outcome was found in carriers receiving standard care compared to non-carriers at post-intervention ($\beta = 9.35$, $p = 0.036$, Table S4). Similarly, VCS carriers in the standard care group also improved less than non-carriers at post-intervention ($\beta = 14.86$, $p = 0.017$, Table S4). No significant association was found in the SSGT group (Table S4).

We also tested the interaction of PRS for ASD from our previous study (Li et al., 2020) and VCS/VUS in the linear model. Outcomes at post-intervention and follow-up were measured by SRS changes at two time points compared to pre-intervention. Similarly, we found carriers of VCS had less improvements at post-intervention (VCS: $\beta = 14.66$, $p = 0.03$). No significant associations were found either in the two-way (VCS/VUS*PRS) or the three-way interactions (VCS/VUS*PRS*interventions), demonstrating the independent effect of PRS and rare pathogenic exome variants on treatment outcomes (Figure S6).

ASD-related gene sets on intervention outcomes

Based on our earlier findings of synaptic gene sets for the intervention outcome (Li et al., 2020), as well as enriched gene sets shown to be significant in a previous large scale genetic study on ASD (Satterstrom et al., 2020), we

TABLE 1 Detailed information of the prioritized clinically significant variants identified in the cohort

Gene symbol	Chr	Position	Reference allele	Alternative allele	Genotype	Effect	Transcript ID	Exon	CDS position change	Protein change	Transcripts affected/ Total transcripts	Allele frequency (gnomAD)
ASH1L	1	155,307,491	C	CT	Het	FRAMESHIFT	ENST00000392403	28/28	c.8854-8855insT	p.R2952X	2/3	4.60×10^{-05}
CHD1	5	98,262,076	ACT	A	Het	FRAMESHIFT	ENST00000284049	1/35	c.13-14del	p.S5X	1/1	-
CUL3	2	225,376,185	C	A	Het	STOP GAINED	ENST00000264414	6/16	c.C769A	p.E257*	4/6	-
MAPK8IP3	16	1,808,160	GGTGA	G	Het	SPLICE DONOR	ENST00000250894	9/31	-	-	2/2	9.63×10^{-05}
KANSL1	17	44,249,260	AGAGAT	A	Het	FRAMESHIFT	ENST00000262419	2/15	c.245-249insGAGAT	p.YL82-83X	7/9	-
PACS1	11	65,838,072	C	T	Het	STOP GAINED	ENST00000320580	1/24	c.C115T	p.Q39*	1/8	-
TBL1XR1	3	176,765,162	G	A	Het	STOP GAINED	ENST00000430069	9/16	c.G790A	p.Q264*	2/14	-
ZBTB20	3	114,058,002	AG	A	Het	FRAMESHIFT	ENST00000462705	10/10	c.1856del	p.P619X	7/9	-
NFIA	1	61,543,298	A	G	Het	START LOST	ENST00000407417	1/11	c.A1G	p.M1V	1/13	-

Note: All coordinates were based on human reference genome build GRCh37/hg19. The transcripts were based on Ensembl GRCh37.
Abbreviations: CDS, coding sequence; Chr, chromosome; Het, heterogeneous.

TABLE 2 Association between clinically/uncertain significant variants and intervention outcomes in the whole cohort

	Beta	Lower CI	Upper CI	p-value	Partial explained variance (η^2)
VCS + VUS					
*SSGT	−1.45	−21.41	18.52	0.89	8.69×10^{-05}
*Post-intervention	9.22	−0.25	18.70	0.057	0.01
*Follow-up	7.56	−2.68	17.80	0.15	6.65×10^{-03}
*SSGT*Post-intervention	−12.16	−25.98	1.66	0.086	9.50×10^{-03}
*SSGT*Follow-up	−10.92	−25.47	3.63	0.14	6.88×10^{-03}
VCS					
*SSGT	3.85	−36.88	44.59	0.85	1.49×10^{-04}
*Post-intervention	14.84	1.60	28.09	**0.029**	0.02
*Follow-up	6.27	−8.67	21.20	0.41	2.19×10^{-03}
*SSGT*Post-intervention	−21.51	−48.89	5.88	0.12	7.78×10^{-03}
*SSGT*Follow-up	−22.77	−51.02	5.48	0.12	8.15×10^{-03}
VUS					
*SSGT	−3.92	−27.37	19.53	0.74	4.69×10^{-04}
*Post-intervention	4.36	−8.04	16.77	0.49	1.54×10^{-03}
*Follow-up	8.09	−4.95	21.12	0.22	4.75×10^{-03}
*SSGT*Post-intervention	−6.63	−23.08	9.81	0.43	2.02×10^{-03}
*SSGT*Follow-up	−8.86	−26.03	8.30	0.31	3.30×10^{-03}

Note: The statistically significant p-value is marked in bold.
Abbreviations: CI, confidence interval; VCS, variant of clinical significance; VUS, variant of uncertain significance.

generated a pilot scheme to calculate gene set specific scores based on both rare (rare deleterious exome variants and rare CNVs) and common variants from ASD-related gene sets which we called GSASD and assessed their effect on intervention outcomes. Protein coding genes expressed in the brain were used as a background gene set. Detailed rare and common variants number for ASD-related and brain expressed genes are listed in Table S5. We calculated GSASD for synaptic transmission genes (GSSyT), regulation of transcription from RNA polymerase II promoter (Pol II) genes (GSPol), developmental disorder-related genes, and genes from SFARI (Data S1) for each individual to represent the genetic load of rare and common variants separately. Common variants GSSyT ($GSSyT_c$) had no association with the intervention outcomes in the whole cohort (Table S6). However, the effect of SSGT compared to standard care at post-intervention was significantly better among individuals with higher $GSSyT_c$ ($\beta = -5.52$, $p = 0.033$, Figure 3). We found a significant improvement at follow-up in individuals with higher rare variant GSSyT ($GSSyT_r$) in the whole cohort ($\beta = -5.38$, $p = 0.016$, Table S6). Interestingly, the effect of SSGT compared with standard care was smaller at follow-up for individuals with higher GSSyTr ($\beta = 8.30$, $p = 0.0044$, Figure 3). There were no significant associations between the intervention outcomes in the whole cohort and common variants GSPol ($GSPol_c$) or rare variants GSPol ($GSPol_r$). However, we found positive effects on SSGT outcome compared with standard care effects for those individuals with higher $GSPol_r$ both at post-intervention ($\beta = -6.38$, $p = 0.018$, Figure 3) and follow-up ($\beta = -6.79$, $p = 0.014$, Figure 3). No significant associations were found for genetic scores of developmental disorder related and SFARI genes and intervention outcomes (Figure 3). After multiple testing, the significance remained for the association of $GSSyT_r$ and $GSPol_r$ with intervention outcomes (Table S6).

To further test the relationship between common and rare variants in GSSyT and GSPol, we also used linear regression adding the interaction effect of rare variants and common variants together with interventions using SRS changes of post-intervention and follow-up. Similar to previous results, differential effects of both $GSSyT_r$, $GSSyT_c$ and $GSPol_r$ were indicated on intervention outcomes (post-intervention: $GSSyT_c$*SSGT: $\beta = -5.95$, $p = 0.028$; $GSPol_r$*SSGT: $\beta = -5.69$, $p = 0.037$; follow-up: $GSSyT_r$: $\beta = -6.67$, $p = 0.014$, $GSSyT_r$*SSGT: $\beta = 9.32$, $p = 0.0077$; $GSPol_r$*SSGT: $\beta = -6.80$, $p = 0.034$). However, we did not detect any significant interactions between rare and common variants in both sets (Figure S7).

Intervention outcome prediction using machine learning test

Lastly, we investigated whether a potential machine learning (ML) model could predict the response by integrating detailed clinical and genetic information from the

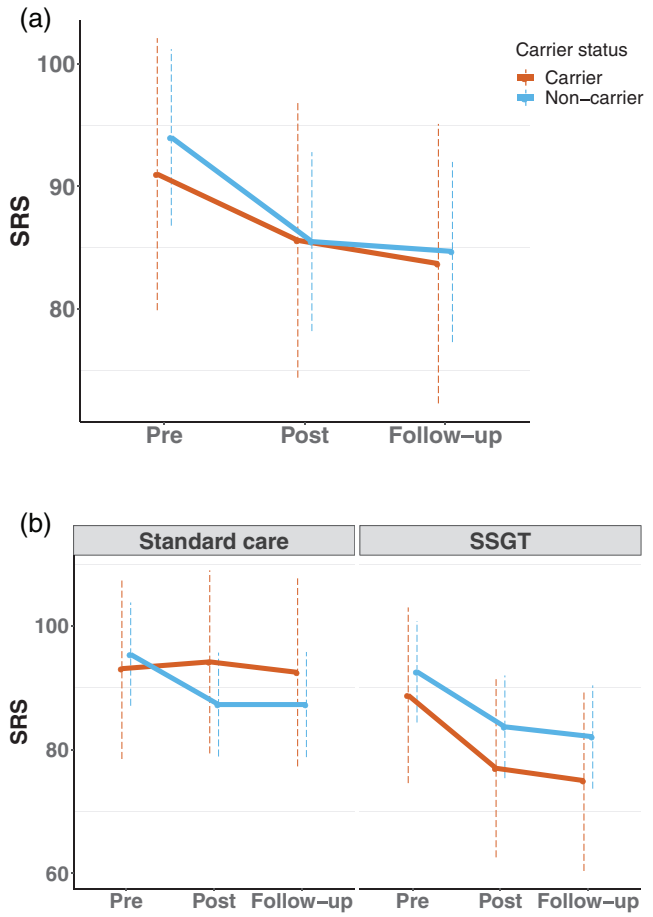

FIGURE 2 Association between VCS + VUS carrier status and intervention outcomes. (a) Least-squares means of social responsiveness scale (SRS) among carriers pre-, post-intervention, and follow-up. (b) Least-square means of SRS among carriers at pre-, post-intervention, and follow-up in standard care and social skills group training (SSGT) groups. VCS, Variant of clinical significance; VUS, Variant of uncertain significance

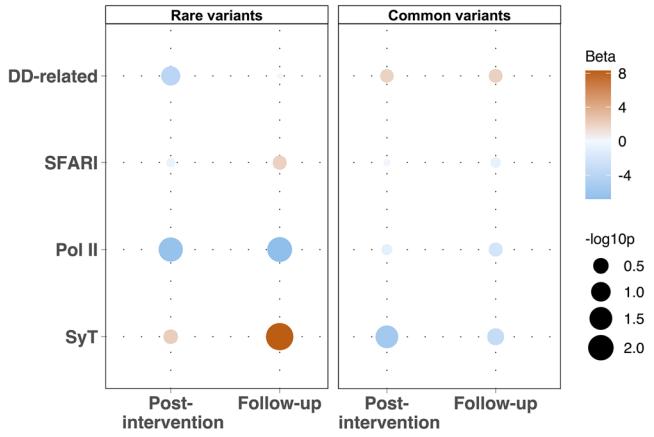

FIGURE 3 The effect of rare and common variant genetic scores for synaptic transmission genes (SyT, GO:0007268), regulation of transcription from RNA polymerase II promoter genes (pol II, GO:0006357), Simons Foundation autism research initiative (SFARI) genes, and developmental disorder-related (DD-related) genes on intervention outcomes at post-intervention and follow-up. The color of each circle represents the coefficient value in the 3-way interaction of $GSSyT_r/GSSyT_c$*time points*interventions. The size of each circle is the coefficient -log10P value calculated from the mixed linear model

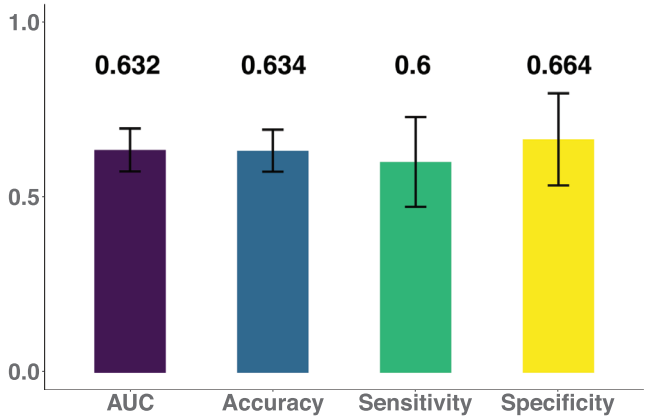

FIGURE 4 Performance of machine learning model measured by area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, and specificity

individuals in the KONTAKT® trial. Participants were separated into two classes based on if they had responses to the interventions (n = 88 [47.3%]) or not (n = 98 [52.7%]) measured by parent-reported SRS at post-intervention. After evaluation, we applied linear support vector machine (Data S1) on selected twelve features, including individual comorbidities, SSGT treatment, SRS and Clinical Global Impression (CGI) score, rare exome variants (number of rare damaging/developmental disorder-related variants), rare CNVs (number of genes in rare CNVs, rare CNV size) and $GSSyT_r$, and used 10% decrease in SRS total score as a binary outcome (Figure S8, Data S1 [Alternative models]). The average area under the receiver operating characteristic curve (AUC) and accuracy was 0.632 (SD 0.0599) and 0.634 (SD 0.0610, Figure 4). The ML model was able to correctly predict individuals' responses to interventions with an average of 60% (SD 0.128) sensitivity and 66.4% (SD 0.131) specificity (Figure 4).

DISCUSSION

Most of the current interventions yield variable responses among autistic individuals. There is minimal knowledge of whether genetic information can guide clinicians to individualize intervention decisions. A few case studies (Cucinotta et al., 2020; Pini et al., 2014; Serret et al., 2015) and our earlier reports indicate a potential to identify an association between an intervention outcome and genetic information (Li et al., 2020; Tammimies et al., 2019). Despite large-scale exome sequencing studies in ASD, no studies have directly connected ASD-related rare sequence variants to intervention outcomes. Here, we investigated whether the carrier status of rare ASD-related sequence variants, categorized as VCS or

VUS, and genetic scores calculated from genes within ASD-related gene sets, influences outcomes following SSGT and standard care from a recent clinical trial. After variant prioritization and the association test, we found that individuals with VCS or VUS together showed limited social behavioral change across the entire cohort and further analysis pinpointed that this effect was especially evident for the standard care group. Furthermore, we showed that this effect could be more prominent for changes in the social cognition domain as demonstrated by a subscale of the SRS. As the psychometric properties of the subscales in SRS are not well established, these results should be interpreted with caution. Additionally, the limiting effect of VCS was stronger than VUS in both the entire cohort and the standard care. As SSGT was an add-on intervention to the standard care in the KONTAKT® trial, the non-significant difference between carriers and non-carriers in the SSGT group might indicate a potentially beneficial effect of SSGT for carriers compared to standard care alone, which should be explored further. Earlier, we demonstrated that individuals with clinically significant CNVs had better outcomes in standard care and less improvements after SSGT (Tammimies et al., 2019). Interestingly, we further showed that biological associations of the intervention outcomes as we tested rare variants genetic scores for synaptic transmission (GSSyT) and regulation of transcription from RNA polymerase II promoter (GSPol). Further our ML model confirmed the importance of rare variants for outcome prediction and provided a feasible pattern for investigating the role of both clinical and genetic features on ASD-related treatments.

We also provided evidence that intellectually able autistic individuals carried genetic variants of clinical significance, as we detected that a minority of 4.4% individuals had VCS, which is within the range of other clinical exome sequencing studies (Guo et al., 2019; Iossifov et al., 2014; Tammimies et al., 2015). Although the proportion is slightly lower than in earlier reports, it is important to note that these studies also included individuals with co-occurring intellectual disability, which is associated with an increased probability of VCS findings (Guo et al., 2019; Iossifov et al., 2014; Tammimies et al., 2015). Combining clinically significant CNVs with the exome sequencing findings, the overall proportion of identifiable genetic etiology of autistic individuals in this cohort was 12.6%, demonstrating that this group of autistic individuals should also have access to clinical genetic testing. As no overlap was found between VCS and clinically significant CNV carriers, our results confirmed the complementary role of exome sequencing and chromosome microarray in increasing the molecular diagnostic yield for individuals with ASD.

We demonstrated nine VCSs from different autosomal-dominant genes implicated in ASD and other NDDs. For example, variants from *CUL3* (OMIM* 603136) have been observed in individuals with pseudohypoaldosteronism (MIM*614496) and contribute to ASD, social deficits, and anxiety-like behaviors (Boyden et al., 2012; Dong et al., 2020; O'Roak et al., 2012). Likewise, we found both clinically significant CNV and exome VCS affected *ASH1L* (OMIM*607999) in our samples related to intellectual disability (MIM*617796), ASD, and Tourette syndrome (Liu et al., 2020; Tammimies et al., 2015). This gene encodes a histone methyltransferase that associates with the transcribed region such as *Hox* genes, catalyzes H3K36 methylation, and plays an important role in neurodevelopment (Okamoto et al., 2017). Additionally, results from rare variants GSSyT and GSPol, although showing an inconsistent impact on interventions, may reveal complicated pathway functions and connections influencing the outcome. The latest randomized controlled trial has indicated that bumetanide that reverts the polarity of GABA responses may help a subset of autistic people with a subset of autism traits (Sprengers et al., 2020), suggesting the synaptic signaling is related to the treatment efficacy. Due to limited power in the sample size, we chose only gene sets/pathways that are highly confirmed in ASD and indicated in our previous study (Li et al., 2020); future studies are required for a more in-depth exploration of ASD-related genes and pathways.

When assessing the distribution of rare and common variants in this cohort, we found that carriers of pathogenic variants and non-carriers had identical levels of PRS for ASD, which is in line with a recent study using a much larger cohort showing that the contribution from common genetic variants is similarly relevant to autistic individuals with or without a strongly acting de novo mutation (Weiner et al., 2017). In addition, Niemi et al. compared PRS between children with severe NDDs (e.g., global developmental delay, intellectual disability, cognitive impairment or learning disabilities, and ASD) with and without pathogenic variants and found no significant differences for any tested PRSs (Niemi et al., 2018). All these results suggest PRS and rare variants associated with ASD or other NDDs may contribute additively to the individual liability for the conditions. Similarly, we found no interaction effect of PRS for ASD and exome pathogenic variants on interventions. As a similar independent role for PRS and clinically significant CNV was shown earlier (Li et al., 2020), our study indicated an additive or differential role for each type of variant contributing to the treatment outcomes.

There is a high genetic and clinical heterogeneity in ASD. Besides traditional association tests, an ML model can integrate measurements to recapitulate useful information to predict intervention outcomes. Using the KONTAKT® trial data, we built a prediction model to select the most contributing features to the intervention responses, including variables representing the complexity and severity of the disorder (comorbidity status, SRS and CGI scores), intervention conditions (SSGT intervention), and genetic liability especially rare variant

related characteristics. These selected features from different domains could provide a prioritized list for future ASD investigations and more evidence for precise guidance into clinical practice of intervention decisions. When the linear model, especially the linear SVM, combined selected variables into outcomes, it outperformed the non-linear model showing 63.4% accuracy, illustrating the outcome was to a certain degree predictable instead of by chance (50%) to help clinicians decide whether an individual could have a response or not after adding SSGT as an add-on of standard care. Due to limited power, our study was inadequate to make predictions for each intervention subgroup. As no study has combined clinical and genetic factors to predict the outcomes of ASD interventions, the performance of prediction in our study could be considered a reference to compare after similar studies become available. Further possible ways to improve the prediction performance include but are not limited to increasing the sample size with more available individual data, adding related clinical evaluations, and refining different levels of genetic factors to explain more variance of outcome changes. After increasing the power with sample size, individuals could be divided into suitable subgroups to predict intervention effects under certain conditions and different levels of response.

Limitations in our study should also be noticed. To begin with, although our study was based on the largest randomized controlled trial of SSGT in ASD to date, limited sample size restricted the power to identify intervention-associated genes and to stratify variant effect in subgroups. Exome sequencing studies of ASD have shown that both de novo and inherited rare variants are major components of genetic risk and are associated with cognitive phenotypes such as IQ (Iakoucheva et al., 2019). However, due to the lack of parental information, our study could not distinguish de novo and inherited rare variants and seek out any differences between variant types. Furthermore, as the intervention methods are varied for ASD, we could not combine samples from other clinical cohorts and validate our findings. Although there are some completed and ongoing clinical trials testing the SSGT effect, exome sequencing data on the same cohorts are unavailable at present (Freitag et al., 2016; Gates et al., 2017). Therefore, without further validations, our results must be interpreted with caution. With increased genetic testing in clinics, converting genetic information into primary diagnosis and treatment, combined with clinical observation and biometric data, could proactively shape clinical decisions and long-term management (Katsanis & Katsanis, 2013). Factors from different aspects should be considered when leveraging genetic data into practice, such as data interpretation, genetic counseling, ethical consideration, and economic constraints (Horton & Lucassen, 2019; Katsanis & Katsanis, 2013). Based on the KONTAKT® clinical trial, we used gene-by-invention interactions to understand why individuals respond differently and uncover the benefits of SSGT or standard care for those at differing levels of genetic risk. With more genetic information acquired from individuals, this approach can be widely applied to understand whether behaviors or other intervention programs varied for individuals with a different genetic vulnerability.

In summary, we have demonstrated that exome sequencing can identify molecular diagnoses in an ASD cohort without intellectually disabled individuals, and genetic information reveals significant associations with intervention outcomes, although with variable effects in the SSGT and standard care groups. We also show that gene set based genetic scores could be further explored to determine meaningful subgroups and direct future studies on biological mechanisms. By developing an ML model, we confirm that individual outcomes could be predicted by clinical and genetic factors such as rare variants. Our study is a preliminary exploration of rare variants and a combination of different level variants on ASD intervention outcomes. Future clinical trials of different interventions in ASD should include genetic data collection to improve the use of molecular genetics beyond diagnoses for individualized intervention plans.

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CONFLICT OF INTEREST

Sven Bölte is an author of the German and Swedish KONTAKT manuals and receives royalties from Hogrefe publishers. Dr. Bölte discloses that he has in the last 3 years acted as an author, consultant, lecturer for Medice and Roche. Nora Choque Olsson and Ulf Jonsson are authors of the Swedish KONTAKT manuals. Martin Becker is a full-time employee of Bayer AG, Germany. The other authors do not report financial interests or potential conflicts of interest.

AUTHORS CONTRIBUTIONS

Danyang Li and Kristiina Tammimies conceived and planned the present study. Danyang Li designed and performed the analyses with supervision from Kristiina Tammimies. Hong Jiao, and Nina Norgren provided overall suggestions and discussion for the analyses. Martin Becker and Abishek Arora performed experiments of prioritized variants for Sanger validation. Sven Bölte and Nora Choque Olsson conceived the original clinical trial. Sven Bölte is responsible for the clinical data and provided the expertise of the clinical measures together with Nora Choque Olsson, and Ulf Jonsson. Danyang Li and Kristiina Tammimies wrote the paper with input from all authors. All authors approved the final version of the article.

ETHICS STATEMENT

The study was approved by the ethical review board in Stockholm (Dnr 2012/385–31/4) and the clinical authorities participated in the clinical trial.

DATA AVAILABILITY STATEMENT

All public data from databases and studies were described in the manuscript. The raw genetic data is accessible from the corresponding author after necessary clearances. Code related to variants prioritization and developmental disorder-related variants selection is available at: https://github.com/DanyangLi107/vcf_varselect. Code related to machine learning test is available at: https://github.com/DanyangLi107/ml_kontakt. All statistical analyses were performed in R v3.6.3. Additional software and package information were illustrated in the Subjects and Methods and Supplemental Information.

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