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ARTICLE

Return of genetic research results in 21,532 individuals with autism

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ABSTRACT

Purpose: The aim of this study is to identify likely pathogenic (LP) and pathogenic (P) genetic results for autism that can be returned to participants in SPARK (SPARKforAutism.org): a large recontactable cohort of people with autism in the United States. We also describe the process to return these clinically confirmed genetic findings.

Methods: We present results from microarray genotyping and exome sequencing of 21,532 individuals with autism and 17,785 of their parents. We returned LP and P (American College of Medical Genetics criteria) copy-number variants, chromosomal aneuploidies, and variants in genes with strong evidence of association with autism and intellectual disability.

Results: We identified 1903 returnable LP/P variants in 1861 individuals with autism (8.6%). 89.5% of these variants were not known to participants. The diagnostic genetic result was returned to 589 participants (53% of those contacted). Features associated with a higher probability of having a returnable result include cognitive and medically complex features, being female, being White (versus non-White) and being diagnosed more than 20 years ago. We also find results among autistics across the spectrum, as well as in transmitting parents with neuropsychiatric features but no autism diagnosis.

Conclusion: SPARK offers an opportunity to assess returnable results among autistic people who have not been ascertained clinically. SPARK also provides practical experience returning genetic results for a behavioral condition at a large scale.

The names of The SPARK Consortium members will appear at the end of the article.

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Introduction

Autism is a heterogeneous neurodevelopmental condition characterized by difficulties with social communication, restricted and repetitive behaviors, and often sensory sensitivities.[1] Population and twin studies have shown that autism is highly heritable[2-4] and that its genetic architecture includes a combination of rare and common coding variants. Gene discovery studies have reported 60 to 102 individual risk genes with exome-wide significance and predict hundreds more.[5,6]

The genes and pathways associated with autism overlap with related conditions, including intellectual disability (ID), epilepsy, and developmental delay (DD). Studies suggest that some genes primarily lead to autism rather than to ID[5,7]; however, these types of analyses are challenging because of differences in phenotyping and ascertainment across cohorts.[8] Other studies suggest no autism-specificity among genes identified either from autism or ID/DD cohorts.[9]

SPARK data have contributed significantly to gene discovery and insights into autism's genetic architecture, including identifying new autism genes with moderate effect size.[6,7,10] SPARK has also committed to returning genetic findings related to autism[11] consistent with consensus recommendations.[12] The aim of this study is to document which participants have a genetic finding that meets American College of Medical Genetics (ACMG) criteria for pathogenicity and can be currently returned to participants as causative of their autism diagnosis. We call these findings returnable results and refer to the rate of return to participants as returnable yield.

Research studies that return results directly to participants mainly return medically actionable variants recommended by the ACMG. Studies doing this at a large scale include eMERGE, All of Us, the Geisinger MyCode community health initiative, and the Million Veterans Program (Vassy JL, Brunette CA, Yi T, et al. Introducing return of results in the Million Veteran Program: design and pilot results of the MVP-ROAR Familial hypercholesterolemia Study. medRxiv. 2023:2023.2010.2007.23295899).[13-17]

A few research studies also directly return autism-related results to participants: MyCode identified and returned copy-number variants (CNVs) linked to neuropsychiatric conditions and autism, including in participants who did not have an associated diagnosed phenotype.[18] The Deciphering Developmental Disorders study has sequenced and analyzed 13,449 individuals with severe developmental disorders and placed nearly 4500 potential diagnoses into personal health records, allowing local clinical result confirmation and return.[19] The MSSNG database has analyzed 5100 individuals with autism by genome sequencing and returns results to participants if they meet clinical standards.[20] The Alabama Genomic Health initiative, a state-funded approach to provide genetic testing, has returned neuropsychiatric variants to a rare disease cohort referred by health care providers.[21]

For most neurodevelopmental and autism-related genetic results, there are no established interventions that change the clinical outcome. However, studies of participants show that etiological explanations are validating and help them understand their condition and life experiences.[18,22] A genetic diagnosis provides access to genetically defined family groups, research studies, and clinical trials.[23] A study of SPARK participants has shown that receiving genetic results related to autism in minor children reduces feelings of guilt in parents and has some impact on their actions and life planning.[24] All results are returned to participants with free, expert genetic counseling or by the participant's health care provider.[25]

Autism's genetic and phenotypic heterogeneity are well documented. However, autism studies reporting the diagnostic yield from exome sequencing (ES) have been largely limited to individuals referred by medical geneticists or neurologists. These studies report yields that range from 13% to 26%.[26-30] Their cohorts tend to have relatively high proportions of ID and structural congenital anomalies. For example, in one study, 93% of 163 individuals with autism sequentially referred for clinical diagnostic ES also had ID.[28] In contrast, only 27% of the individuals with autism in SPARK endorse ID or DD. Furthermore, only 6.8% of people with autism in this study disclosed having had chromosomal microarray analysis, and 1% reported ES, despite clinical recommendations for genetic evaluation.[31]

We identified a returnable result in 8.6% of SPARK participants with autism, with higher frequency among participants with other medical features, such as motor delay or large head size. We also find results across the phenotypic spectrum of autism, including in transmitting parents with neuropsychiatric features but no autism diagnosis. We describe the processes needed to return individual research results at scale, including challenges returning both de novo and inherited genetic results to minors, dependent adults and independent adults, and challenges with transition to adulthood in a longitudinal cohort.

Materials and Methods

Participants and data

SPARK uses diverse recruitment strategies to recruit participants online and in-person at clinical sites across the United States.[32] If participants opt in to the genetics portion of the SPARK study, they receive a saliva kit in the mail (or through their clinical site). A single guardian for each dependent indicates their consent and their dependent's assent (≥10 where capable) and preferences for receiving genetic results. All study activities are approved by a single, central institutional review board (Western Institutional Review Board- Copernicus Group board protocol #20151664). When participants reach the age of majority, their consent expires. Parents are sent a guardianship form to indicate if they remain legal guardians, which restores consent. If parents report that their child is now an independent adult, they may invite the child to reregister and consent for themself.

Participants and their family members completed self- or parent-report questionnaires to collect behavioral data and comorbidities. Participants or clinical sites mailed saliva samples to a Clinical Laboratory Improvement Amendments (CLIA)/College of American Pathologists (CAP)-certified clinical genetic laboratory (Exact Sciences) for DNA extraction, and aliquots of DNA were sent to a research laboratory for ES. ES data were processed as previously described.[6]

Because race and ethnicity were not originally surveyed during registration, this information is available for 76% of the autism cohort in this analysis. To increase statistical power, participants were grouped as White (endorsed White as race), or non-White (endorsed any category other than "White," including "other" and "more than one"). Participants who endorsed being Hispanic were grouped according to their separate responses for race. Genetic ancestry for each individual was assigned using common, high-quality single-nucleotide polymorphisms (PLINK—maf 0.05—geno 0.1) available in both SPARK and HapMap3.[33] Using principal component analysis, each SPARK participant was assigned an ancestry based on a normalized likelihood vector of each sample belonging to each HapMap3 population. If 2 classes were identified, African was assigned over European, American over European, American over east Asian, South Asian over European, and American over African. Others were assigned unknown. Unknown assignments were not included in the analysis.

For the analysis on transmitting parents, we used diagnoses from a medical history screening questionnaire, completed by 88% of participants with autism (or their guardians). Endorsing a developmental diagnosis includes ID, cognitive impairment (CI), global DD, or borderline intellectual functioning; language delay or language disorder; learning disability; motor delay; mutism; social pragmatic communication disorder; or speech articulation problems. Endorsing a mood or anxiety condition includes anxiety disorder, bipolar (manic-depressive) disorder, depression or dysthymia, disruptive mood dysregulation disorder, obsessive-compulsive disorder, or hoarding. The analysis also looked at attention-deficit/hyperactivity disorder (ADHD), schizophrenia, and seizures.

All statistics were performed using R (/R Suite) using the general linearized model for logistic regression.[34] Univariate regression was performed using the gt_summary package, which uses the general linearized model function, and P values were adjusted with Bonferonni correction (Figures 1 and 2).[35] P values for all proportions in the figures were based on a 2-value z-test of proportions from the rstatix package in R.[36]

Variant calling

Variant calling was done using GATK HaplotypeCaller (v4.1.2.0), weCall (v2.0.0), and DeepVariant (v.0.8.0). Per-sample gVCFs were jointly called with GLnexus (v1.1.3). De novo single-nucleotide variants (SNVs), de novo (DN) insertion-deletion mutations (indels) and rare SNVs and indels were identified as previously outlined in Zhou et al,[6] using 2 distinct sets of final filters.

CNVs were called independently by several pipelines that used calls from CoNIFER (v0.2.2), xHMM (v 1.0), and CLAMMS (v 1.1) on the ES data and support from PennCNV(v1.0.4) and CRLMM (v1.38.0) calls on genotyping arrays.[6] We reported high-confidence merged rare CNVs (defined as those occurring in less than 3% of families within the cohort).

Variant selection

We implemented a systematic and objective set of criteria for determining which genetic findings were returnable in SPARK. Variant analysis for SNV_INDELS was limited to 487 unique, dominant-acting, or X-linked genes (Supplemental Table 1). Genes were included if they were annotated by ClinGen Expert Panels as having a strong or definitive association with ID and autism, epilepsy, rasopathies, or Rett and Angelman-like conditions (230 genes).[37,38] Genes were also included if they were annotated by the PanelApp as meeting strong or definitive associations for neurodevelopment, autism, and/or ID, based on disease name or Human phenotype ontology terms (257 genes).[39]

Loss-of-function (LoF) variants in genes classified as "absent mutation consequence" in the above databases were included. (LoF variants in the last exon of the protein were considered if other variants distal or 3' to this variant, have been classified as pathogenic in the literature). LoF variants in genes annotated as "altered mutation consequence" were returned if there was published evidence of pathogenicity. Missense variants met ACMG criteria for likely pathogenic or pathogenic (LP/P).[40]

SPARK also returned aneuploidies (trisomy 21, pathogenic partial chromosome duplications or deletions, X, XXY, XYY, and any sex chromosome aneuploidy leading to 48 chromosomes) and recurrent, well-established CNVs and other rare CNVs meeting ACMG likely pathogenic/pathogenic criteria. For recurrent CNVs, 51 recurrent CNVs annotated by the Dosage Sensitivity Expert Panel with sufficient evidence for haploinsufficiency ($n = 38$) or triplosensitivity ($n = 13$) for a neurodevelopmental condition were included, as well as any LP or pathogenic CNV using the ClinGen CNV Interpretation Calculator.[41]

The list of returnable genes/variants is updated along with the ClinGen and PanelApp databases and changes are approved by a committee of medical geneticists. Participants could be notified about a returnable result immediately after genomic data analysis or in subsequent reanalyses as the returnable gene/variant list changed.

Variant confirmation and interpretation

All results that SPARK aimed to return to participants were independently confirmed and interpreted by a

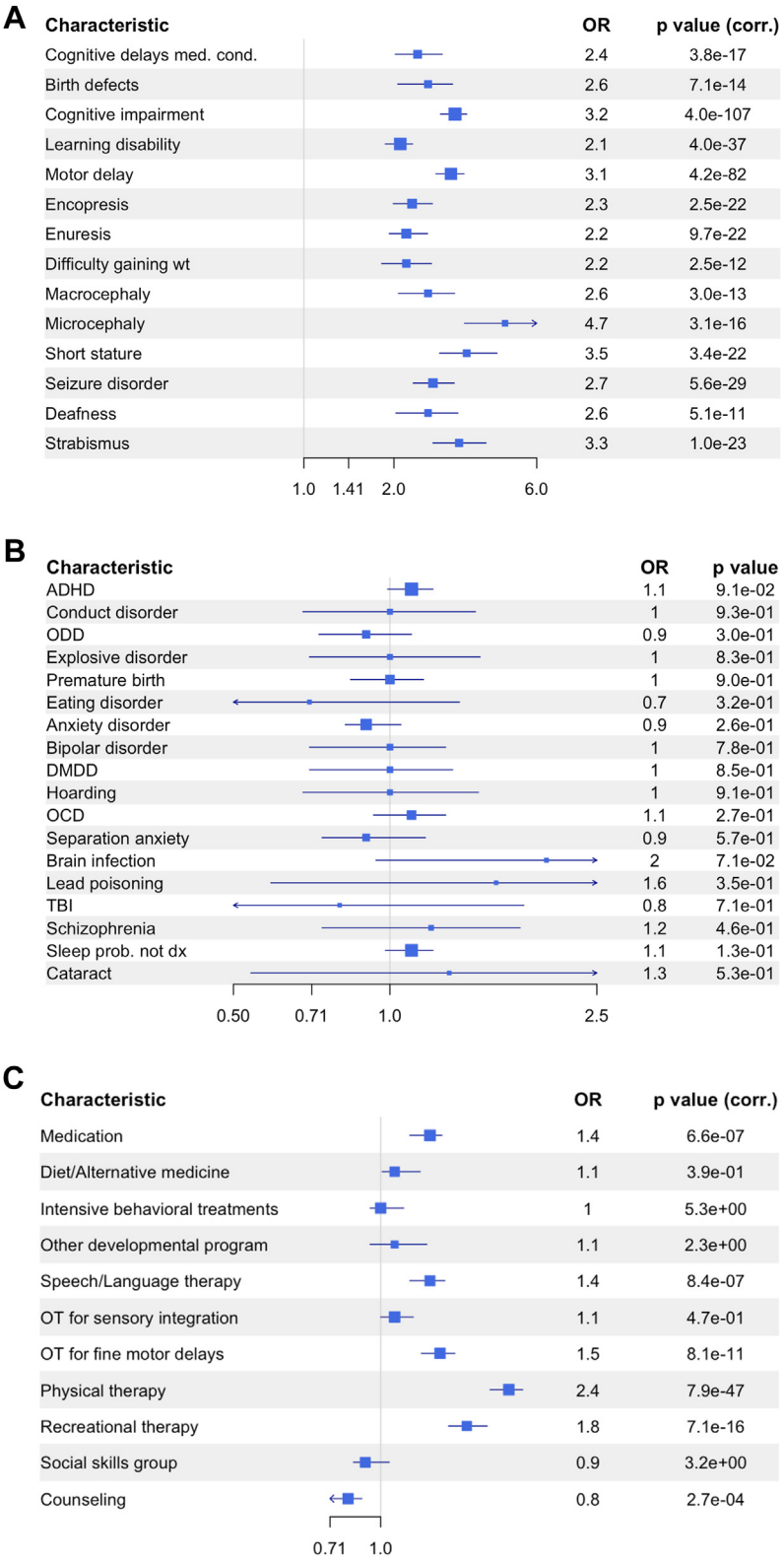

Figure 1 Features that increase the odds of having a likely pathogenic/pathogenic result for autism. A. Univariate logistic regression performed on 51 individual features endorsed in the basic medical questionnaire completed by 88% of participants with autism (participants who did not complete this questionnaire are excluded from this analysis). Significant features with an effect size (OR) greater than 2 and which remained significant after correction for multiple testing, false discovery rate, are listed. Univariate regression was performed using the gtsummary package, which uses the glm function in R, with "having a result" as the model outcome and individual features as model covariates.[36] False discovery rate for multiple testing was performed and q-values for each of the 51 features are listed in Supplemental Table 3. Each *P* value displayed in 1A is Bonferroni corrected by adjusting for the 51 original features. cog. delays. med cond.,

CLIA/CAP-certified laboratory using orthogonal methods on a banked DNA sample stored in the CLIA/CAP laboratory. Confirmations were performed in the individual with autism and any available parents and, if not presumed de novo, in any other available first-degree relatives with autism. Any variants interpreted as "variants of uncertain significance," "benign," or "likely benign" were excluded from the analysis and not returned to participants. Results reported as already known to participants and results in participants who were no longer eligible for return (eg, reached age of majority and did not yet reconsent) were not clinically validated.

Workflow for returning results

SPARK informed eligible participants of any LP/P result for autism by email, letter, and phone call. To receive their result, participants needed to log into their account on the SPARK website and complete an online 2-question release of information/authorization form in line with privacy practices. This form allows SPARK to send the result either to the participant's medical provider or to a SPARK-provided board-certified genetic counselor. This authorization step (step 1) allowed participants to confirm that they still wanted to learn about a genetic result at the time that it became available for return. Participants then scheduled a call with a SPARK-provided genetic counselor, or their own provider, to learn of the specific result (step 2).

In the case of inherited variants, parents without autism who transmitted a genetic result to their child with autism were sent an additional consent form compliant with NYS-79-L before they were contacted about a result for themselves. (Each family member and an equal number of participants without results received this consent form, to avoid revealing a result for return).

SPARK contacted participants with autism who do not have a returnable result after SNV and CNV analysis annually by email. SPARK invited eligible participants with a genetic result from SPARK to Simons Searchlight, a genetics-first, international, online research registry for ~200 genetic conditions associated with autism. Continued participation in SPARK may include invitations to genetics-first research studies and/or clinical trials. The workflow of the basic steps of return of genetic results in SPARK is shown in Figure 3A.

Results

Study cohort

The majority of participants with autism in this analysis (83%) were minors at the time of enrollment, 8% were independent adults, and 9% were dependent adults enrolled by a parent (Table 1). The ratio of males to females with autism was 3.3:1, which is consistent with 2020 US prevalence estimates of 3.8:1.[42] Registered participants under 18 years of age had a male ratio of 3.9:1, whereas those over 18 had a ratio of 1.7:1. Participants with autism in this cohort had a median registration age of 9 years; 8 for children and 24 for adults, who ranged up to 77 years of age. The median age at diagnosis for participants with autism was 3.8 years; 4.4% of SPARK participants with autism reported that they received their autism diagnosis as adults.

Overall, 27% of participants with autism endorsed CI or ID, global DD, or borderline intellectual functioning as diagnoses. However, 49% of participants with autism endorsed symptoms of DD/CI, which include parent-estimated cognitive-level, language-level, and motor deficits[6]; 12% of participants with autism had at least one of the following features: congenital anomalies, seizures, macrocephaly, or microcephaly. Out of 16,247 participants with autism who provided race and ethnicity data, 78% self-identified as only White (see Table 1 for breakdown).

Return of results to SPARK participants

Participants in this study are 21,532 individuals with autism and 17,785 of their parents. Of SPARK participants who submitted saliva, 99.3% elected to receive genetic results related to autism. A total of 1906 LP/P autism-related results were identified in 1861 participants (Supplemental Table 2). Of these results, 1666 were not previously known by the participant. Of these unknown results, 36% of these could not be validated because of insufficient DNA and/or participant reaching the age of majority and not yet reconsenting and 6 participants did not consent to result return when registering. (SPARK sends additional kits to participants with insufficient DNA and regularly checks to see if participants who turned 18 have returned to the study as either dependent or independent adults. A small fraction of participants contacted responded.) The remaining 1116 participants with

cognitive delays due to medical condition; Diff. gaining weight, difficulty gaining weight. B. Subset of features from the univariate analysis for which the CI interval crosses 1. Dis., disorder; DMDD, disruptive mood dysregulation disorder; OCD, obsessive-compulsive disorder; ODD, oppositional defiant disorder; Prem. Birth, premature birth; Sep. anxiety, separation anxiety; Sleep prob. not dx, sleep problems not diagnosed; TBI, traumatic brain injury. C. Univariate logistic regression on the endorsed use of interventions, from an additional optional questionnaire, completed by 76% of participants with autism. Regression was performed as in 1A, with result as the model outcome and intervention-type as the model covariate. *P* values listed are Bonferonni corrected for the 11 interventions tested.

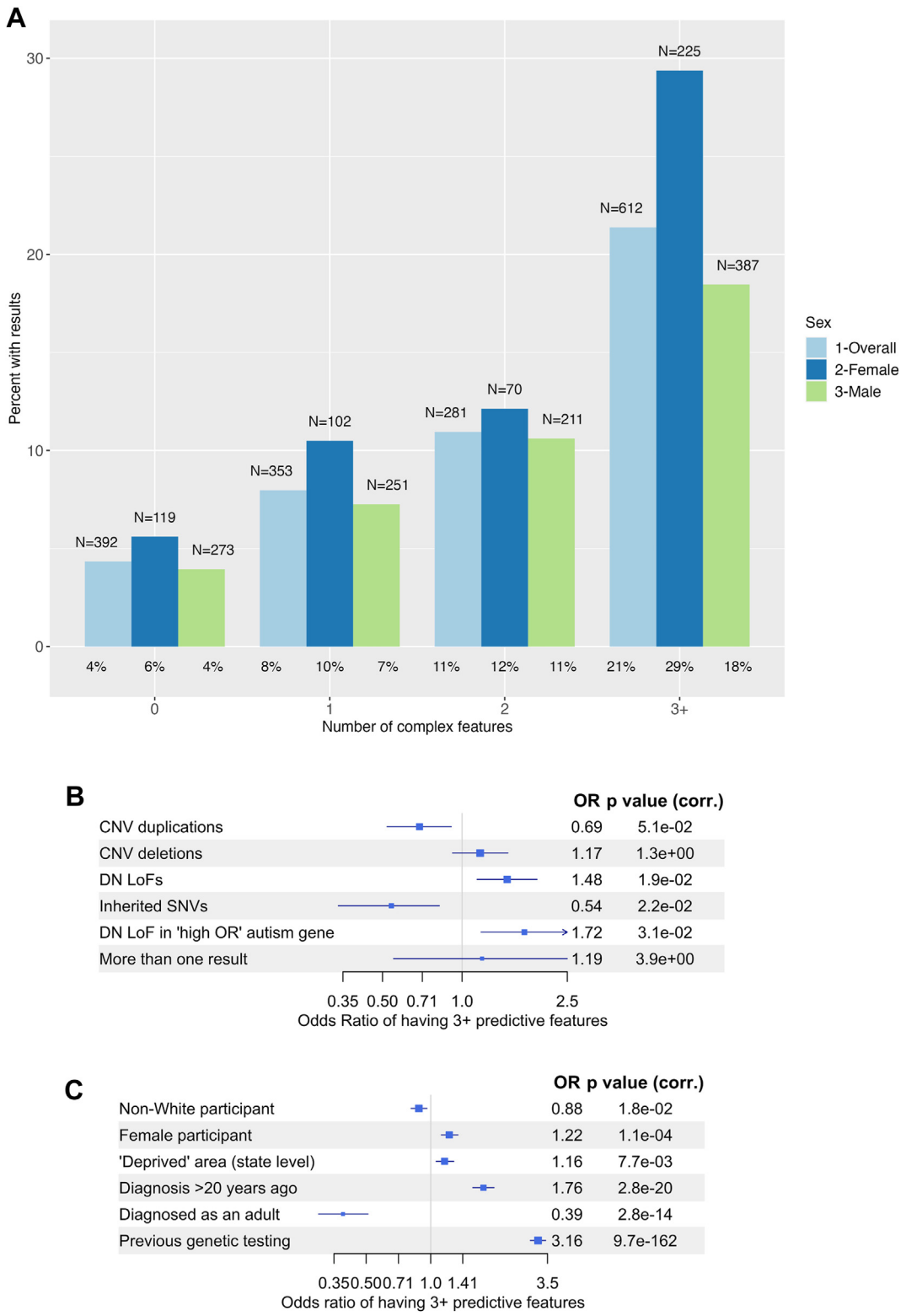

Figure 2 Predictive features increase diagnostic yield, are associated with stronger-impact variants, and are enriched in certain demographic groups. A. Participants were grouped based on the number of predictive features they endorsed that increased the rate of result return by an odds ratio (OR) of 2 or greater and were statistically significant from 1A. Result yield in all participants and female and male participants is displayed. B. Univariate regression was performed on whether having 3+ predictive features (from 1A) increases the likelihood of carrying a certain type of result. Regression was performed as in 1A, with 3 or more predictive features as the model outcome and mutation type as the model covariate. *P* values listed are Bonferroni corrected for 6 comparisons. DN loss of function in "high-OR" autism gene refers to a gene with an OR of 10 or greater in Rolland et al. C. Univariate regression was performed on what demographic features increase the likelihood of having 3+ predictive features (from 1A). Regression was performed as in 1A, with 3 or more predictive features as the model outcome and participant characteristics as the model covariate. *P* values were corrected with Bonferroni correction for 6 comparisons.

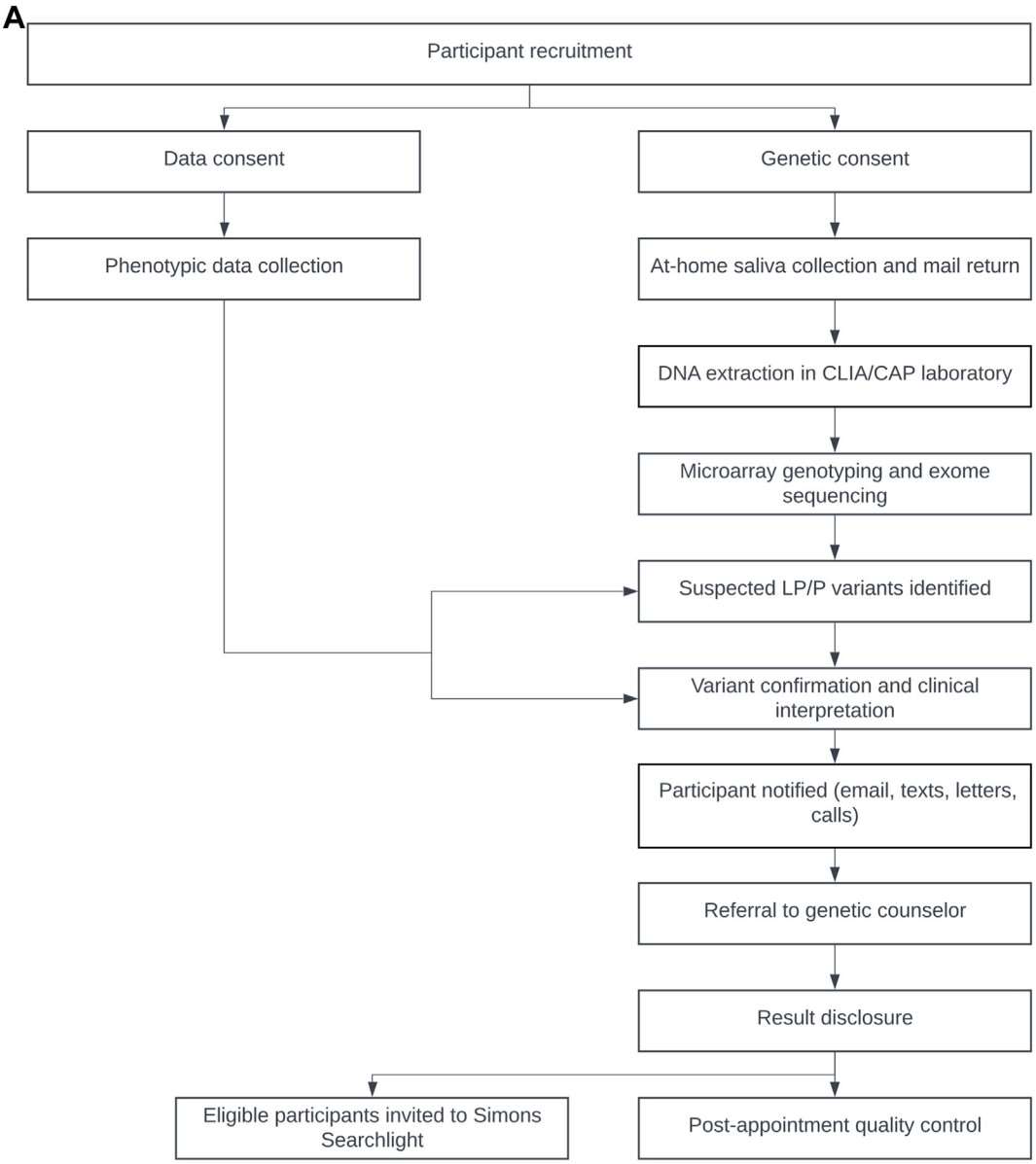

Figure 3 SPARK result return protocol. A. Operations workflow leading to the return of likely pathogenic/pathogenic autism results in SPARK participants with autism. B. Return status for the 1861 likely pathogenic/pathogenic autism results described in the study. Return status is ongoing and was locked at time of article submission.

confirmed LP/P variants were contacted about potential results, and fewer than 1% actively declined.

To receive their results, participants needed to complete 2 steps (see Materials and Methods). Of the contacted participants, 62% completed the first step, and 85% of those participants went on to complete the second step. A total of 12% of contacted participants did not complete the first step after several communication attempts, and 4% became ineligible upon reaching the age of majority before completion (16% are still in the multistep contact process). Of the contacted participants, 7% completed the first step but did not schedule an appointment with a genetic counselor within 6 months or did not attend their scheduled appointment. (Figure 3B).

Returnable findings

The returnable yield for an autism genetic result (LP/P autism variant) in this analysis was 8.6% ($N = 1861/21{,}532$) in all people with autism, 11.7% ($N = 588/4418$) in females and 7.7% ($N = 1273/15253$) in males. The odds of females having a result were 1.6 times ($P = 8.4$ e−19, 95% CI: 1.4-1.8) greater than males. The male/female ratio for participants with a LP/P autism result is 2.2:1 (Figure 4A).

Of all results, 54% were SNVs or indels in AD genes, 4% were SNVs in X-chromosome genes, 36% were CNVs, and 4% were chromosome aneuploidies (Supplemental Table 2). Nine participants had homozygous or compound

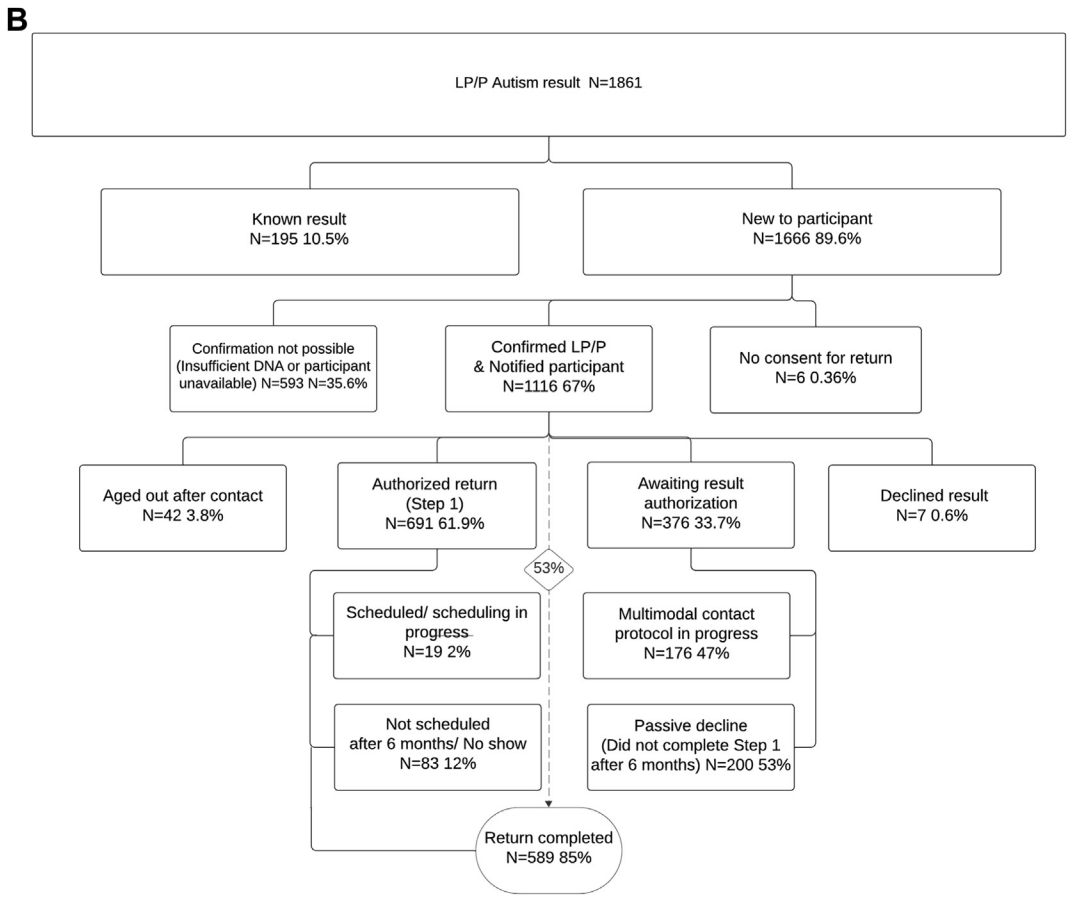

Figure 3 Continued.

heterozygous recessive variants linked to autism, based on an analysis of 9804 individuals with autism who were in the first SPARK sequencing release. Thirty-five participants had more than 1 result (excluding recessive findings). Excluding recessive conditions and aneuploidies, the most common result type was de novo SNVs (29%) followed by SNVs of unknown inheritance (25%) (Figure 4B).

There were 448 individual genetic events, meaning a gene with LP/P variants, a recurrent CNV, or an aneuploidy. Of these 448 events, 186 (42%) were observed in only 1 individual in the cohort, and 81% were seen at most 5 times.

Of the 1861 individuals with a result, 3.1% (58) carry a deletion that overlaps the 16p11.2 (BP4-BP5 recurrent region (seq[GRCh38] 16p11.2(29638676_30188531)x1), and 3% (55) carry a duplication that overlaps this same region (seq [GRCh38]16p11.2(29393947_30203920×3). The next most common CNVs are duplications that overlap the 15q11.2 BP-BP3 recurrent region (seq[GRCh38115011.2(23465365-28134728)x3) in 2.3% (43) of individuals with results and deletions that overlap the 15q13.3 (BP4-BP5) (seq[GRCh38] 15q13.3(30900686-32153204)x1) in 2% (36). The most common autism LP/P SNV/INDELS were 23 LoF and 11 missense variants in *SCN2A* (HGNC:10588), 27 LoF and 6 missense variants in *SHANK3* (HGNC:14294), and 19 LoF and 11 missense variants in *PTEN* (HGNC:9588) (Figure 4C). The prevalence of most common finding, 16p11.2 deletions, was 0.3% (58 of 21,532 participants with autism).

At the variant level, the most common LP/P recurrent variants were NP_000305.3:p.(Arg335Ter) in *PTEN* in 6 participants/4 families, NP_001269461.1:p.(Tyr719Ter) in *ADNP* (HGNC:15766) in 4 individuals/4 families, and NP_542168.1:p.(Asn308Asp) in *PTPN11* (HGNC:9644) in 4 individuals/3 families. All 3 variants were pathogenic in ClinVar.

Predictive phenotypic features

We performed a univariate logistic regression on the association between 51 features from the medical questionnaire and the likelihood of having a returnable result. Features with an odds ratio (OR) greater than 2 that remained significant after correction for multiple testing are shown in Figure 1A. The full univariate regression and the proportions of each feature in the cohort are listed in Supplemental Figures 1 and 2. Features with no effect on the likelihood of having a returnable result (confidence interval spans 1) are displayed in Figure 1B. Overall, symptoms of CI and medical features such as motor deficits, congenital anomalies and atypical head size increased the odds of an

Table 1 Cohort demographics

Variable	*N*	Overall, *N* = 21,532[a]	Age at Registration (y)		(Missing), *N* = 114[a]	*P*-Value[b]
			<18, *N* = 17,885[a]	>18, *N* = 3533[a]		
Sex	21,532					<.001
Male		16,526 (77%)	14,218 (79%)	2229 (63%)	79 (69%)	
Female		5006 (23%)	3667 (21%)	1304 (37%)	35 (31%)	
Registration age (y)	21,418	9 (5, 14)	8 (5, 12)	24 (20, 32)	NA (NA, NA)	<.001
Diagnosis age (y)	21,418	3.8 (2.5, 6.6)	3.5 (2.5, 5.6)	8.0 (3.3, 19.3)	NA (NA, NA)	<.001
Endorsed race	16,247					
White		12,735 (78%)	10,599 (77%)	2136 (83%)	0 (NA%)	
More than one		1,724 (11%)	1,513 (11%)	211 (8.2%)	0 (NA%)	
African American		754 (4.6%)	663 (4.8%)	91 (3.6%)	0 (NA%)	
Other		607 (3.7%)	541 (4.0%)	66 (2.6%)	0 (NA%)	
Asian		346 (2.1%)	301 (2.2%)	45 (1.8%)	0 (NA%)	
Native American		64 (0.4%)	52 (0.4%)	12 (0.5%)	0 (NA%)	
Native Hawaiian		17 (0.1%)	16 (0.1%)	1 (<0.1%)	0 (NA%)	
Cognitive impairment	19,355	5306 (27%)	4207 (26%)	1085 (34%)	14 (100%)	<.001
Medically complex[c]	18,939	2230 (12%)	1722 (11%)	501 (16%)	7 (13%)	<.001

[a] *n* (%); Median (IQR).
[b] Pearson's χ^2 test; Kruskal-Wallis rank sum test; Fisher's exact test.
[c] Participants termed "medically complex" endorsed at least one of congenital anomaly, seizures, and macro- or microcephaly.

individual having a returnable genetic result. By contrast, mental health diagnoses (such as ADHD or anxiety), and environmental factors (such as premature birth) were not associated with a returnable genetic result.

In a separate analysis, we performed a univariate regression on the association between having a returnable autism result and endorsing the use of 10 different interventions from an additional optional questionnaire completed by 76% of participants (Figure 1C). Among the interventions, physical therapy, recreational therapy, and occupational therapy were most associated with results. Together, the results suggest that returnable results are associated with more complex medical presentations.

We refer to features associated with a result with an OR of 2 or greater (from Figure 1A) as predictive features. These features are cognitive delays/impairment, learning disability, microcephaly, macrocephaly, short stature, strabismus, congenital anomalies, motor deficits, seizures or epilepsy, low-weight, and inability to control bowels or bladder past developmentally expected age. Of participants with 3 or more predictive features, 21% had results, and 392 participants (4%) had a returnable result despite having none of these predictors. Adjusting for sex, having 3 or more predictive features increased the odds of a result by 3.9 times, (CI: 3.5-4.4 $P = 3.8$ e−132, (Figure 2A).

We tested the hypothesis that the total number of predictive features correlates with the type of genetic result. We found that having 3 or more predictive features increased the likelihood of a de novo LoF, and this association increased when variants were restricted to genes previously identified as having an effect size in autism of 10 or greater.[43] There was no statistically significant association between predictive features and all DN SNVs nor with all DN events. In contrast, the presence of 3 or more predictive features decreased the likelihood of having a CNV duplication or an inherited SNV (Figure 2B), suggesting that predictive features are associated specifically with variants of stronger, rather than those of more moderate, effect.

We performed an additional OR analysis to see whether certain groups of participants are more likely to have this predictive phenotype. Significant positive findings included being female, living in a neighborhood that is above average for deprivation based on the area-deprived index (state level) (ADI = 6+),[44] receiving an initial autism diagnosis more than 20 years ago, and having had previous genetic testing. Significant negative findings include having had a first diagnosis as an adult (Figure 2C).

Participants from presumed simplex families had a slightly higher return frequency than other families (9.1%, OR 1.3, CI: 1.2-1.5 $P = 1.2$ e−05) when controlling for the proportion of trios (individuals who were presumed simplex did not endorse having a first-degree family member with autism and did not have another enrolled family member with autism). When the analysis was restricted to females with 3 or more predictive features from simplex families ($N = 437$), the returnable result frequency increased to 32%, (OR: 5.4, CI: 4.4-6.6 $P = 3.1$ e−57).

Neither the father's nor the mother's age at the time of the birth of a participant with autism were significantly associated with receiving any returnable finding.

Returnable yield

Returnable yield varied based on several demographic factors. The odds of participants receiving a result decreased (OR: 0.78, CI: 0.72-0.84, $P = 1.1$ e−11) for every 10 years increase in the age of diagnosis (aka age 4 versus 14). The odds of

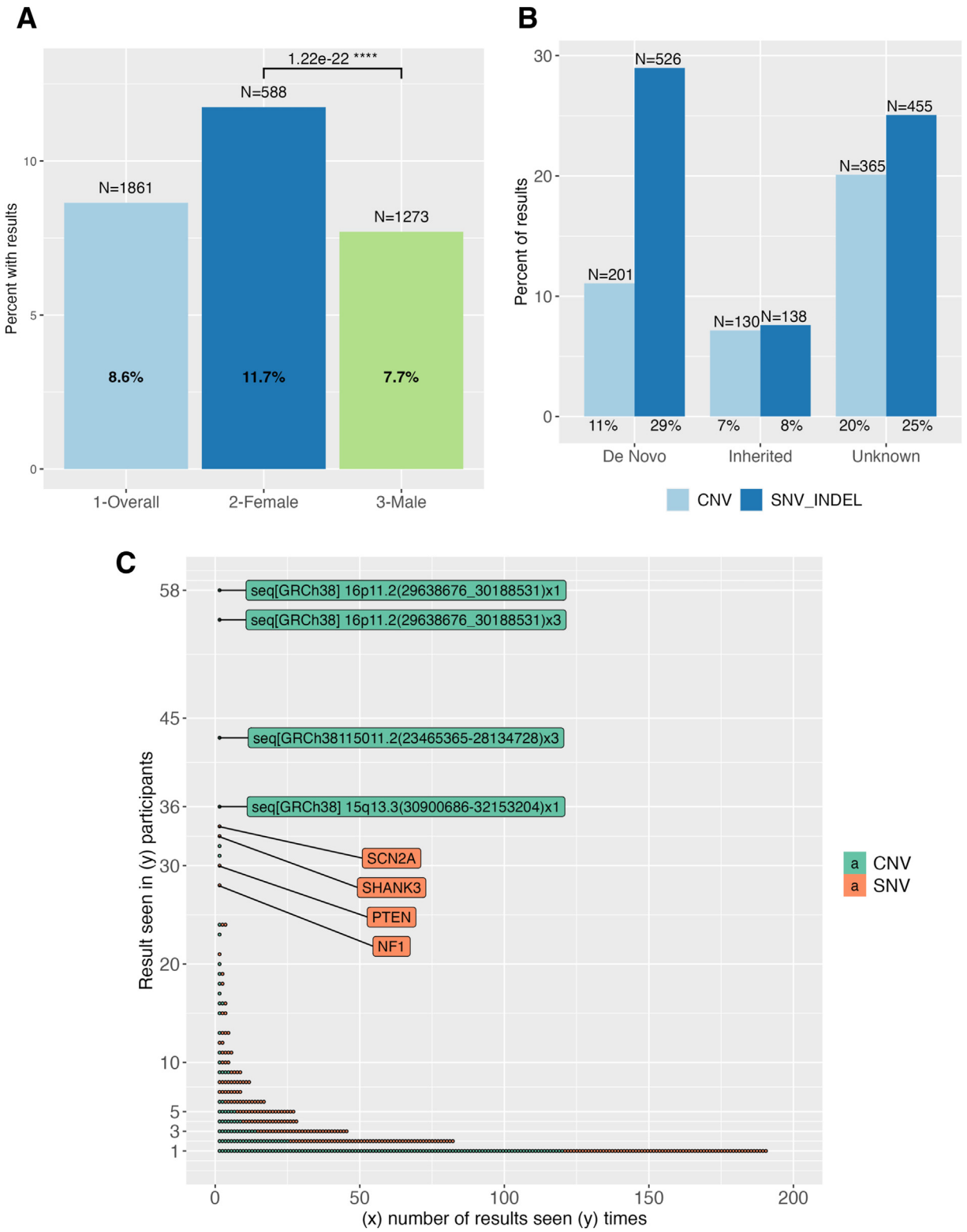

Figure 4 **Profile of likely pathogenic/pathogenic genetic results in SPARK.** A. Rate of result return in 21,532 participants with autism, 1861 of whom carry a total of 1903 variants (44 individuals have more than 1, including 9 individuals with homozygous and compound heterozygous recessive variants). Significance between males and females is 1.2 e−22 on a *z*-test of proportions, with an OR of 1.6 CI: 1.4-1.8 $P = 8.4$ e−19. B. Excluding recessive conditions and aneuploidies, the most common result type was de novo SNV_indels (29%) followed by SNV_indels of unknown inheritance. C. 448 genes with likely pathogenic/pathogenic variants, overlapping copy-number variants (CNVs) that map to the same regions, or chromosomal aneuploidies were mapped on the *y*-axis according to the number of participants with these events. The *x*-axis shows the number of results seen with the recurrence rate displayed on *y*. For labeled CNVs, each individual CNV variant overlaps the indicated recurrent coordinates. Individual variant coordinates are listed in Supplemental Table 2.

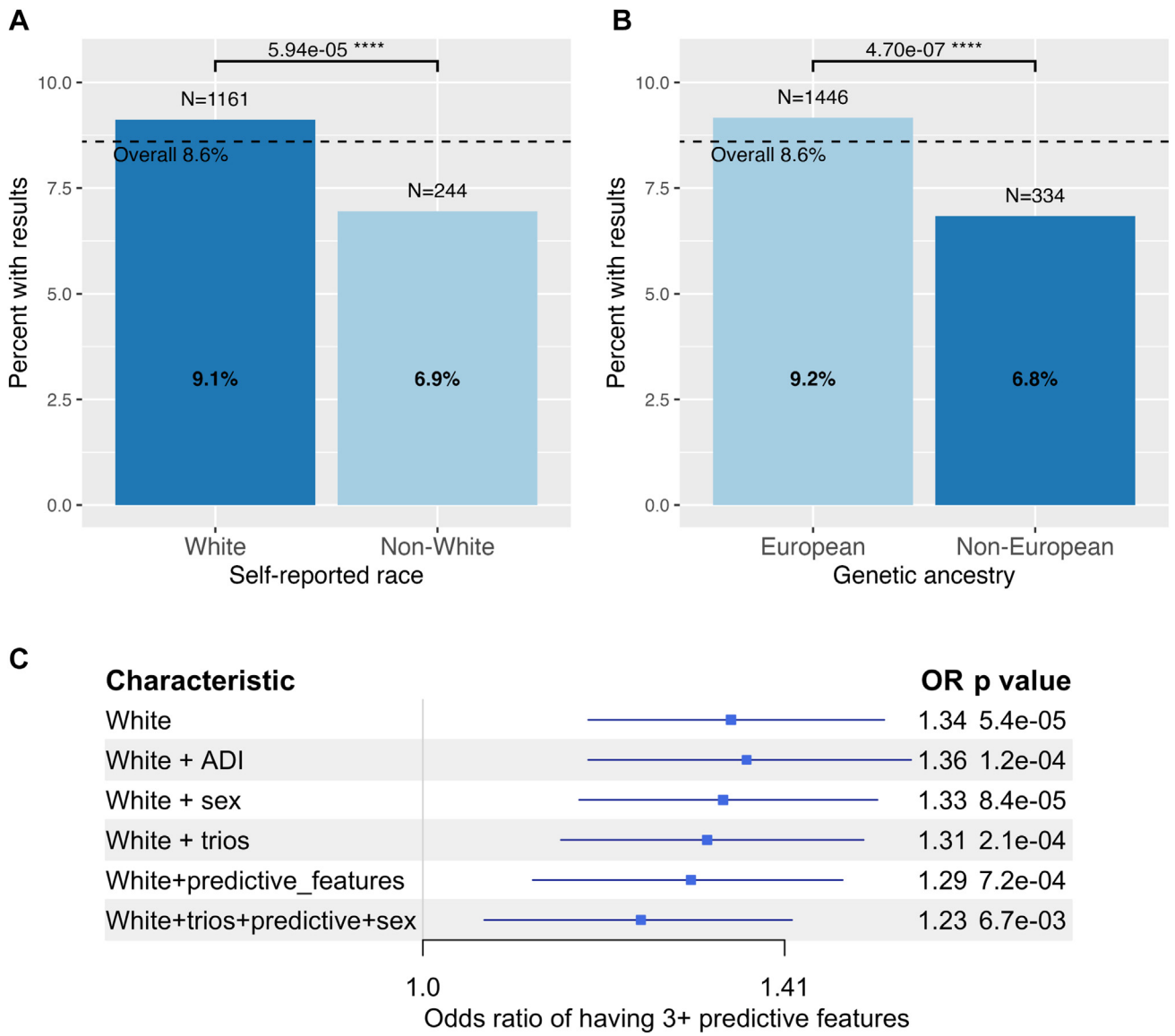

Figure 5 Non-White participants receive a lower rate of result return due, in part, to ascertainment differences. A. Result yield in non-White versus White participants. Hispanic participants are grouped according to their response for race. Analysis is on 16,247 participants (76% of cohort) who have available race data. B. Participants were grouped into European or non-European genetic ancestry based on the HapMap3 reference data (see Materials and Methods). Non-European ancestries are South Asian, East Asian, African, and American. C. The logistic regression between being self-endorsed as White and having a result in SPARK was adjusted for the following factors: participant sex, whether both parents are enrolled in the study, whether participants have 3+ predictive features (1A), and whether participants reside in a neighborhood with a state area-deprived index >5.

receiving a result for participants diagnosed in adulthood were roughly half that of those diagnosed as children (OR: 0.47, CI: 0.33-0.62, $P = 1.7$ e−06). These adult participants were less likely to have CI (OR: 0.21, CI: 0.16-0.26, $P = 8.0$ e−35) and 3 or more predictive features (Figure 2C) compared with participants diagnosed as children.

Adjusting for having both parents in the study, participants were 1.5 times more likely to receive a result if their initial diagnosis was over 20 years ago, compared with within the past 20 years (CI: 1.3-1.7, $P = 3.5$ e−10). Participants diagnosed more than 20 years ago were also more likely to have CI compared with participants diagnosed in the past 20 years (OR: 2.9 CI: 2.6-3.2, $P = 5.7$ e−115) and more likely to have 3 or more predictive features (Figure 2C) Adjusting for CI, the association of time since diagnosis became borderline significant (OR: 1.2, CI: 1.03-1.4, $P = .02$).

Self-reported race was also associated with having a genetic result: 9.1% of White individuals had an autism-related result compared with 6.9% in non-White individuals (OR: 1.3, CI: 1.2-1.6, $P = 5.4$ e−05) (Figure 5A), and the findings were similar when based on European versus non-European genetic ancestry rather than self-report (Figure 5B). This higher frequency of result return in White

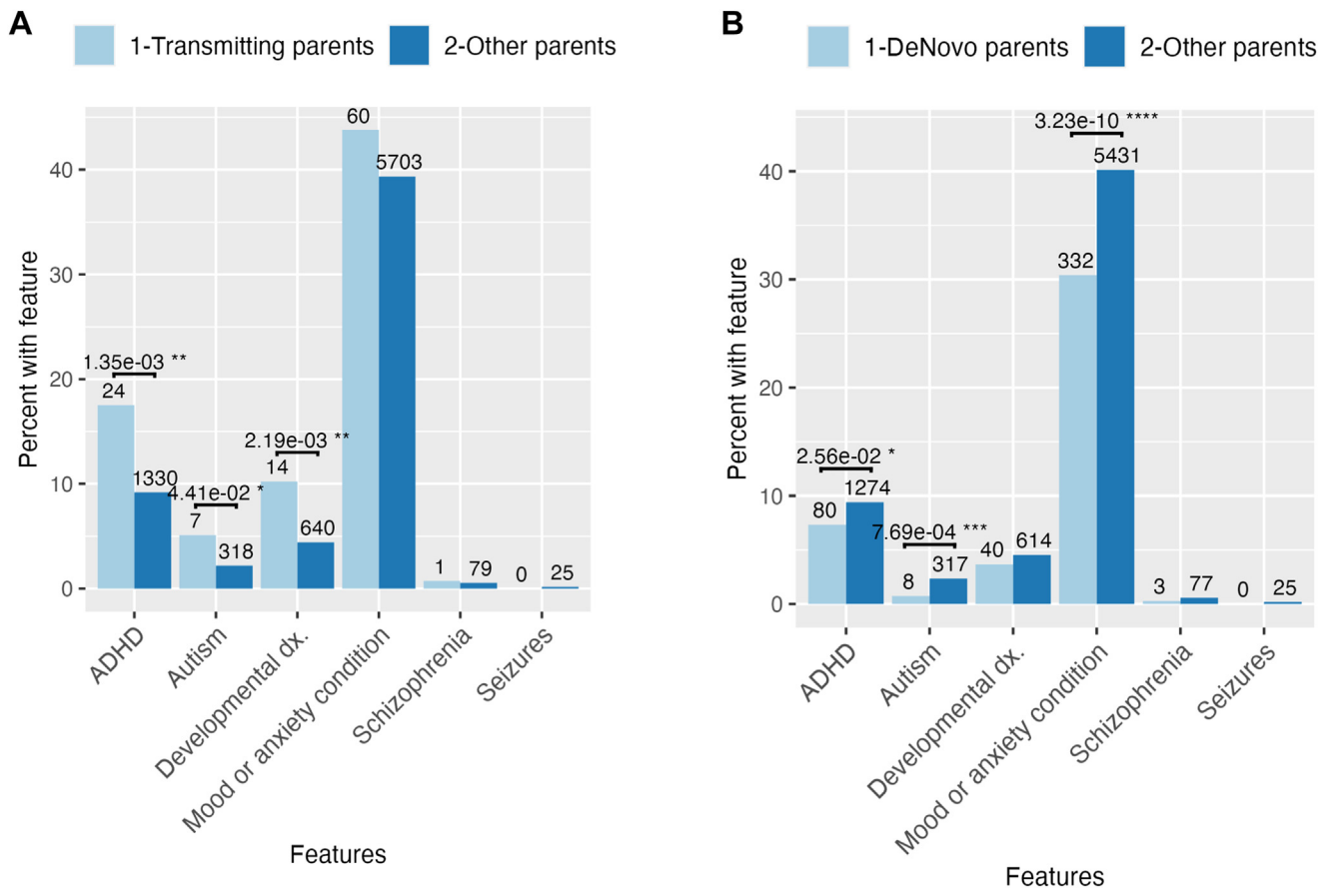

Figure 6 Neuropsychiatric features are enriched in transmitting parents and lessened in parents of children with de novo findings. A. Rates of phenotypic endorsement in 137 parents who transmitted results to children, compared with all other parents of children with autism included in the cohort (14,493). "Developmental diagnosis" is a branching question that includes ID, cognitive impairment, global developmental delay, or borderline intellectual functioning; language delay or language disorder; learning disability; motor delay; mutism; social pragmatic communication disorder; or speech articulation problems. Mental health diagnosis is a branching question that includes anxiety disorder; bipolar (manic-depressive) disorder; depression or dysthymia; disruptive mood dysregulation disorder; obsessive-compulsive disorder or hoarding; and schizophrenia. Parents who endorsed multiple features may be in overlapping groups. B. Rates of phenotypic endorsement in 1092 parents of children who have a de novo genetic finding compared with all other parents of children in the study (13,538). Parents of de novo children are significantly less likely to endorse attention-deficit/hyperactivity disorder and or a mental health diagnosis compared with all other available parents of the cohort.

participants decreased when controlling for availability of parental data, the number of predictive features, and sex (OR: 1.2, CI: 1.1-1.4, $P = .007$) (Figure 5C).

Results in parents without autism

A total of 157 parents transmitted LP/P variants to their children with autism; 137 of these parents completed a basic questionnaire and were included in the following analysis. We examined frequencies of phenotypic features between transmitting parents and nontransmitting parents for ADHD, autism, a developmental diagnosis, a mood or anxiety condition (see Materials and Methods), schizophrenia, or seizures.

Overall, transmitting parents had significantly higher frequencies of ADHD, autism, and developmental diagnoses (Figure 6A). We repeated the analysis in parents whose children had a de novo LP/P variant (1372 total and 1092 with phenotypic data). The results trended in the opposite direction from those for transmitting parents: ADHD, autism, and mood or anxiety conditions were significantly less common in parents of offspring with de novo diagnoses compared with in other parents (Figure 6B).

Discussion

Returnable yield

SPARK identified a returnable result for autism in 8.6% of participants, 5% with SNV variants, 3% with CNVs, 0.4% with aneuploidies, and 0.2% with multiple events. This contrasts with other published studies that identify genetic diagnoses in 13% to 26% of autism cohorts typically

evaluated in genetic or neurology clinics. More than half of the SPARK cohort was recruited online and did not require referral by a clinical provider who had a suspicion of a genetic etiology. As a result, SPARK participation reflects a much broader and more inclusive group of individuals with autism compared with published diagnostic cohorts. When the study sample was restricted to participants who have multiple medical or cognitive features, the yield increased to 21%.

Only 27% of the SPARK study cohort (with autism) endorsed CI and 12% endorsed seizures, congenital anomalies and/or micro- or macrocephaly. By comparison, a 2010 study found a diagnostic yield of 16% from chromosomal microarray and ES in 258 children with autism who were referred from pediatric developmental clinics of whom 30% had multiple physical or congenital anomalies. Notably, the diagnostic yield was only 6.3% in participants who did not have structural anomalies or dysmorphology.[29] A follow-up genome study found a higher proportion of genome-wide rare variants in participants with dysmorphology compared with those without.[45] A yield of 26% was reported for autism samples sent for diagnostic sequencing to a clinical laboratory; 93% of this group had DD, and 39% had seizures.[28]

Having 3 or more predictive features also significantly increased the likelihood that participants had had previous genetic testing, supporting the theory that the subset of SPARK participants with a result is more representative of participants who are currently being referred for genetic testing. Despite the increased utilization of clinical genetic testing in this group, clinical ES was not common: 1% across the cohort with autism and 3.8% in participants with 3 or more predictive features.

In the context of this study, returnable yield refers to genetic results that we can confidently return to participants: at the gene level, they meet ClinGen criteria for a strong or definitive association between the gene and autism, and at the variant level, they meet current ACMG criteria for pathogenic/LP. As such, it does not include moderate effect variants, such as those identified in Zhou et al.,[6] or common variants (polygenic risk), which—although an important part of the genetic architecture of autism—do not fulfill the evidence threshold required to return in SPARK.[46]

Lessons learned from returning hundreds of results

SPARK is one of the largest research studies that aims to return condition-specific genetic results to participants. Returning results related to autism requires navigating autism's genetic heterogeneity, the cognitive disabilities of some of the participants, participants who are often minors, and a range of expectations regarding genetic results. Despite 62% of SPARK participants with autism (or their guardians) endorsing that they believe their autism has a genetic cause, SPARK identified a LP/P result in less than 10%. Participants who suspected their autism has a genetic etiology were only slightly more likely to receive a genetic result from SPARK compared with those who do not suspect a genetic etiology (OR: 1.2, CI: 1.1-1.4, $P = 2.0\ e{-}04$). However, participants who suspected a genetic etiology were 2.3 times more likely to be from a multiplex family compared with those who were not (OR = 2.3, CI: 2.1-2.5, $P = 1.7\ e{-}106$). This suggests that participants may expect genetic results in SPARK to provide an explanation for the heritable nature of autism, which is not reflected in type of LP/P variants routinely currently identified.

The SPARK study also underscores the difficulty in balancing careful result return with participant burden. Only 53% of participants who were told they had a result completed the multiple steps required to receive this information (this percentage rises to 68% if we exclude participants actively being contacted or with a scheduled genetic counseling appointment). A total of 12% of the 691 participants who completed the first step of result return (online authorization) ultimately did not schedule and meet with a genetic counselor within 6 months or did not show to their appointments.

Other studies show that the more steps required from participants to return results the lower the completion rate. For example, the eMERGE network also found a drop-off in participation when additional steps were required for result return (average 75%).[14] A 2021 study of return of secondary findings through the Massachusetts General Brigham Biobank completed return in 52% of 293 eligible participants, with a workflow that required an additional sample for clinical confirmation.[16]

Barriers to return results to participants in SPARK included eligibility that changed over time as minors reached the age of majority, DNA quality, and participant drop-off. The greatest barrier to eligibility is when minor participants reached majority and either the parent did not indicate continued guardianship or the minor did not reconsent as an independent adult. Several research results could not be clinically confirmed because of (saliva) sample quality, especially CNVs that have stringent DNA concentration requirements for validation.

Predictive features

SPARK genetically assessed all participants who provided consented biospecimens, regardless of phenotypic profile. As a result, SPARK provides a unique opportunity to assess phenotypic features associated with returnable results without biasing the cohort toward individuals with a suspected genetic etiology. Our findings are consistent with how clinicians practice, in that associated medical features that suggest CI or a medical condition increase the odds that a participant will have a returnable result.

The higher frequency of returnable results in presumed simplex families is likely related to the criteria for returning results, which prioritize highly penetrant, rare variants that tend to be de novo and/or require fewer familial factors to reach the threshold of autism in the proband. Individuals from

simplex families are more likely to have a de novo finding compared with those from multiplex families (OR: 1.8, CI: 1.4-2.3, $P = 7.5$ e−07). This finding is consistent with data that show reverse correlation between polygenic risk for autism and genetic load of highly penetrant variants.[47]

Cohort factors

Certain demographic factors also increased the likelihood that a participant would have a result. For example, participants who received their autism diagnosis more than 20 years ago were more likely to have a returnable result compared with those who received their diagnosis within the last 20 years, and this was associated with co-occurrence of CI and medical features. This suggests that people diagnosed with autism longer ago may represent a more medically complex cohort compared with individuals diagnosed more recently. These participants with older diagnoses are also less likely to have had access to clinical genomic testing; therefore, this population is both more likely to have a result from genetic testing and less likely to have received genetic care.

In this study, being White increased the odds of receiving a result by 30% over non-White participants; however, adjusting for differences in proportions of trios, female participants, and medical and cognitive features between the groups minimized both the magnitude of the effect and significance of this association. Because SPARK is primarily returning de novo variants, it is unlikely that this disparity is primarily related to ancestry or the ability to identify pathogenic variants within certain groups. A 2023 study showed, for example, that the frequency of diagnostic ES variants identified in pediatric and prenatal populations was not affected by whether participants were part of an underrepresented minority or an underserved group.[48]

In SPARK, non-White participants have slightly fewer associated medical features compared with White participants. One reason for this trend may be that non-White participants with more associated features are more likely than White participants to be diagnosed with an alternative diagnosis such as ID, a phenomenon that is well documented.[49] As a result, non-White participants with medical features may be less likely compared with White participants to enter SPARK with an autism diagnosis.

Phenotypic expansion

Despite the correlation between increased medical features and returnable results, SPARK was able to return LP/P results across the entire cohort, albeit at different frequencies. Of the 392 participants in the study who endorsed none of the predictive features, 392 (4%) nevertheless had a returnable result.

Because of the size and ascertainment of the cohort, SPARK may be able to provide significant phenotypic expansions across autism-linked variants. For example, a 2017 study described 5 individuals with nonsense variants in *IRF2BPL* (HGNC:14282), all of whom showed severe neurodevelopmental regression.[50] The SPARK cohort identified 9 individuals with LoF variants in *IRF2BPL*, 2 of whom are independent adults and 3 of whom inherited the variant from a parent. Variants in another gene, *DYNC1H1* (HGNC:2961), were reclassified from variant of uncertain significance to LP for autism after data including SPARK participants demonstrated the association between this gene and autism;18 individuals in SPARK have variants in *DYNC1H1* that were not returnable at the beginning of the study.

SPARK's scale also reveals unusual findings. For example, we identified a variant in an X-linked gene, *USP9X* (HGNC:12632), in a severely affected male participant, although the condition was previously described as a female-specific form of DD.[51] We returned a diagnosis of phenylketonuria that was biochemically confirmed in a middle-school-age child born in the United States who had not been previously diagnosed or treated.

Strengths and limitations

SPARK is an online study that is open to any US participant who has online access. Despite this, White participants and participants from a higher-than-average socio-economic background are overrepresented. Factors that skew participation likely include, but are not limited to, internet access, access to a reliable autism diagnosis, motivation to participate in and trust in research studies, language, and available time. At the time of recruitment for this cohort, participation was limited to English. Nevertheless, SPARK provides researchers a rich resource of genomic and phenotypic information in tens of thousands of individuals with autism and the potential for recontact and longitudinal follow-up.

Data Availability

Approved researchers can obtain the SPARK data described in this study by applying at base.sfari.org. Analyses were conducted using the SPARK phenotypic data set V7.

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Ethics Declaration

SPARK protocols and communications are approved by a single, central IRB (Western Institutional Review Board-Copernicus Group IRB Protocol #20151664). Informed consent was obtained from all adults, consent was provided by guardians for minors under 10 and assent was obtained for all minors 10 and over, as required by the IRB. All individual data are deidentified.

Conflict of Interest

Wendy K. Chung is on the Board of Directors for Prime Medicine and Rallybio. Jessica R. Wright, Irina Astrovskaya, Sarah D. Barns, Alexandra Goler, Xueya Zhou, Chang Shu, LeeAnne Green Snyder, Bing Han, Yufeng Shen, Natalia Volfovsky, Jacob B. Hall, and Pamela Feliciano declare no potential conflict of interest.

Additional Information

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The SPARK CONSORTIUM

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