The impact of clinical genome sequencing in a global population with suspected rare genetic disease

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iHope provided clinical genome testing to a global population of 1,004 underserved individuals with suspected rare genetic disease. Diagnostic yield, and the proportion with a change of management, was >40% for individuals from both low- and middle-income or high-income countries, suggesting that widespread availability of genomic testing may reduce health disparities.

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The impact of clinical genome sequencing in a global population with suspected rare genetic disease

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Summary

There is mounting evidence of the value of clinical genome sequencing (cGS) in individuals with suspected rare genetic disease (RGD), but cGS performance and impact on clinical care in a diverse population drawn from both high-income countries (HICs) and low- and middle-income countries (LMICs) has not been investigated. The iHope program, a philanthropic cGS initiative, established a network of 24 clinical sites in eight countries through which it provided cGS to individuals with signs or symptoms of an RGD and constrained access to molecular testing. A total of 1,004 individuals (median age, 6.5 years; 53.5% male) with diverse ancestral backgrounds (51.8% non-majority European) were assessed from June 2016 to September 2021. The diagnostic yield of cGS was 41.4% (416/1,004), with individuals from LMIC sites 1.7 times more likely to receive a positive test result compared to HIC sites (LMIC 56.5% [195/345] vs. HIC 33.5% [221/659], OR 2.6, 95% CI 1.9–3.4, $p < 0.0001$). A change in diagnostic evaluation occurred in 76.9% (514/668) of individuals. Change of management, inclusive of specialty referrals, imaging and testing, therapeutic interventions, and palliative care, was reported in 41.4% (285/694) of individuals, which increased to 69.2% (480/694) when genetic counseling and avoidance of additional testing were also included. Individuals from LMIC sites were as likely as their HIC counterparts to experience a change in diagnostic evaluation (OR 6.1, 95% CI 1.1–∞, $p = 0.05$) and change of management (OR 0.9, 95% CI 0.5–1.3, $p = 0.49$). Increased access to genomic testing may support diagnostic equity and the reduction of global health care disparities.

Introduction

Globally there are estimated to be at least 260 million individuals affected by rare diseases, the vast majority of which are genetic in origin and undiagnosed.[1] While there is an increasing recognition of the growing burden of noncommunicable diseases in low- and middle-income countries (LMICs),[2] less attention has been paid to individuals with one of the more than 4,400 well-described rare monogenic diseases.[1] These disorders have a wide range of

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presentations inclusive of epilepsy, developmental or intellectual disability, neuromuscular and movement disorders, structural abnormalities, and primary immune deficiencies.[1] The majority (~70%) present in childhood[1] and are associated with high utilization of healthcare services and unfavorable health outcomes.[3,4] A precision diagnosis provides the basis for care and its absence or delay can result in both missed and inappropriate interventions.[3,4] Recognizing the importance of the availability of molecular testing for precision diagnosis, the World Health Organization Science Council recently stated that it is neither ethically nor scientifically justifiable for a significant delay to exist between the availability of genomic technologies in HICs and their availability in LMICs.[5]

There is increasing evidence for the use of clinical genome sequencing (cGS) as a first-line test for individuals with signs or symptoms of a genetic disease in the pediatric outpatient population,[6–10] but there have been few efforts to improve equity of access.[11,12] The iHope program was established with an aim of providing cGS testing to individuals with limited means and reduced access to molecular testing in resource-limited communities. We previously described a cohort of sixty individuals from a single iHope program site in Tijuana, Mexico.[12] Here we expand upon our prior investigation to describe a cohort of 1,004 individuals drawn from 24 global sites with heterogeneous, suspected rare genetic disorders and report on cGS diagnostic yield and the impact of genomic findings on diagnostic evaluation (DE) and change of management (COM).

Subjects and methods

Reporting was guided using the STROBE checklist for observational cohorts (please see supplemental methods).

Study population and data source

The iHope program is a philanthropic clinical implementation program which, during the time of this investigation, was funded and managed by Illumina Inc. Whole-genome testing was provided through the Illumina Clinical Services Laboratory (ICSL), a CLIA-certified (CLIA# 05D1092911), CAP-accredited (CAP# 7217613) testing laboratory. We performed an observational retrospective cohort analysis of de-identified data derived from 1,004 individuals from 981 families who received cGS testing through the iHope program from June 2016 through September 2021 (Figure 1; Table S1). Individuals were drawn from a network of 24 clinical sites in the United States, Mexico, Peru, the Democratic Republic of Congo, Ghana, Italy, New Zealand, and the United Arab Emirates. Participation in the iHope program was limited to individuals with signs or symptoms consistent with a RGD, without an etiological diagnosis or access to molecular testing.[6,8,13,14] Ordering clinicians included genetics specialists (e.g., geneticists and genetic counselors), non-genetics specialists (e.g., neurologists), and pediatricians practicing in ambulatory clinics or inpatient settings. Each iHope program site participated in an onboarding process which included an overview of the cGS test and its benefits and limitations, possible test outcomes including the possibility of uncertain results, and program eligibility criteria to guide enrollment. Sites were instructed that clinically available genetic testing should be pursued prior to enrollment in the iHope program. Additional education and support were available on request on an ongoing basis through the Illumina Clinical Services Laboratory or iHope program staff. In addition to site-mediated referrals, 26 individuals received cGS through individual clinician request and ordering. The cohort described here includes 60 iHope program individuals which were previously described.[12] For additional details please see the supplemental methods.

The retrospective analysis of de-identified iHope program clinical testing performance, and associated clinical outcomes data, was reviewed by the WIRB-Copernicus Group (WCG) which granted an Institutional Review Board Exemption with a HIPAA Full Waiver of Authorization as defined in US Department of Health and Human Services 45CFR46.104(d)(4). Informed consent for cGS testing was obtained by the ordering clinician at the time of ordering.

Clinical genome sequencing

Clinical GS included interrogation of single nucleotide variants (SNVs), small insertions and deletions (indels), and copy number variants (CNVs) for all samples. Mitochondrial SNVs, bi-allelic absence of the *SMN1* (MIM: 600354) c.840C allele at GRCh37 chr5:70247773 (GenBank: NM_000344.3) (c.840C>T), and short tandem repeat analyses were validated and launched during the study period (supplemental methods and Table S2). Test result categories included: positive, in which a likely pathogenic or pathogenic variant(s) was reported in a disease-associated gene(s) consistent with the clinical presentation and expected disease inheritance pattern; inconclusive, in which variants of potential clinical significance were reported, inclusive of variants of unknown significance; and negative, in which no variants were reported related to the indication for testing. Per laboratory policy, reanalysis was performed when requested by the ordering clinician or when identified during routine laboratory investigations (e.g., a variant reclassification occurred in another individual), and the reanalyzed test result is reflected in the results. As recommended by the American College of Genetics and Genomics, secondary findings reports were optionally provided for all individuals tested (Table S2).[15,16] Ancillary pharmacogenomics screen reports were provided for all tested individuals but were not considered in this analysis (Table S3).

Phenotype distribution assessment

To systematically assess phenotypes, Human Phenotype Ontology (HPO) terms were extracted from clinician-provided indication for testing phenotypes using PhenoTagger.[17] HPO terms were mapped to one of 25 top-level terms by organ system (direct descendants of "Phenotypic abnormality," HP:0000118).[18] To investigate the impact of phenotype on diagnostic yield, the unique number of phenotypic terms and the phenotypic information content of these HPO terms was assessed (supplemental methods).

Ancestry assessment

Principal component analysis of the iHope cohort was performed using genotypes extracted from the cGS data overlayed on a reference set of 3,320 genomes derived from the 1000 Genomes Project, Human Genome Diversity Project, and Simons Genome Diversity Project.[19–21] Admixture was assessed using ADMIXTURE 1.3.0 with a supervised background of the 3,320 individuals from the reference set.[22] For additional details see the supplemental methods.

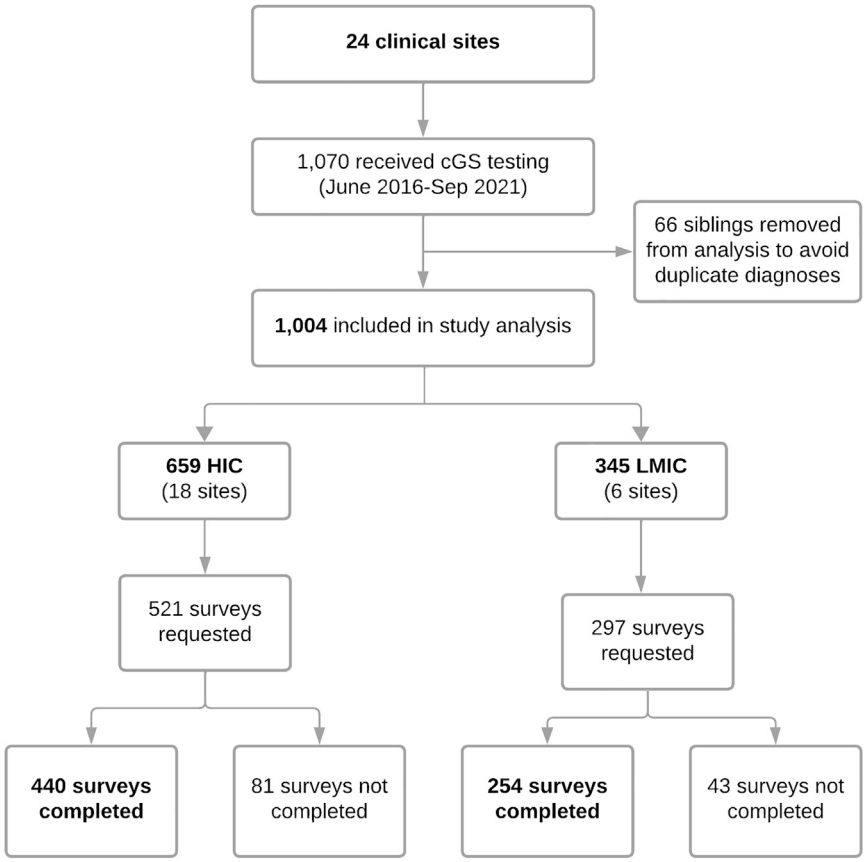

Figure 1. STROBE diagram
iHope program observational cohort ascertainment and cGS impact survey responses depicted using a STROBE diagram. Abbreviations: cGS, clinical genome sequencing; HIC, high-income country; LMIC, low- and middle-income country.

Assessment of cGS test results impact on clinical evaluation and change of management

A cGS impact survey was provided to the ordering clinicians for individuals who pursued cGS from June 2016 through October 2020 (818) (Figure 1). The survey included multiple-choice questions and free-text responses designed to assess the impact of cGS results, regardless of report status, on DE and COM. Three survey versions were used over the course of the study, and total responses available vary by question (Table S4). To ensure consistency of data representation, a subset of responses to the questions regarding impact to diagnostic evaluation and change of management were re-coded from free to text to categorical responses with support of an expert medical geneticist (J. Belmont). As part of data quality control, the survey responses were reviewed for conflict, and when clear conflicts were identifiable, the clinician was contacted. N/A was a response category for change in diagnosis intended to capture scenarios in which cGS findings may be ambiguous (e.g., a VUS of potential clinical significance). Electronic survey responses were received 472 days, on average, after clinical report delivery. For additional details see the supplemental methods.

Outcomes

Diagnostic yield was assessed as the proportion of individuals with a positive test result. A change in DE was defined as the endorsement of any of six categories of clinical assessment. Two delineations of COM were utilized for analysis: a restricted definition which included specialty referrals, imaging and testing, therapeutic interventions, and palliative care; and an extended definition that also considered avoidance of additional testing and genetic counseling. To avoid inflation of estimates of diagnostic yield, secondary and incidental findings were not included in diagnostic yield assessment, but DE and COM survey responses allowed for consideration of these results. For additional details see the supplemental methods.

Statistical analysis

Cohort characteristics

Cohort characteristics including phenotypes, age, sex, and family structure were summarized using descriptive statistics. iHope program sites were categorized based on country of site location according to the World Bank gross national income (GNI) per capita designations: low (<$1,085), lower middle (<$4,255), upper middle (<$13,205), and high (≥$13,205) income (see web resources). The categories low, lower middle, and upper middle were collapsed into a single low- and middle-income country (LMIC) category and compared to HIC.

Outcomes analysis

Observational factors potentially associated with cGS test results, DE, and COM were modeled using a directed acyclic graph to facilitate selection of factors for adjustment when examining outcomes across LMIC and HIC sites (Figure S1). A multinomial regression was performed to assess the impact of LMIC or HIC site designation on test result category. The influence of LMIC or HIC site designation on survey question endorsement was evaluated by multiple logistic regression using a logit link where the survey question determined a binary outcome. The logistic regression included test result category and LMIC or HIC site designation as explanatory variables. The multiplicative influence of LMIC or HIC site designation on the odds of endorsement of DE or COM was estimated by treating HIC as the reference category. A stratified analysis approach was employed to investigate the potential association of LMIC or HIC site designation on survey response outcomes within each test result category. An ANOVA framework was used to compare the number of phenotypic terms submitted per individual between HIC and LMIC sites. Bootstrap resampling was used to compute CIs, and a permutation analysis was performed to determine a p value. These same methods were used to assess the association of phenotypic information with cGS test findings. For additional details please see the supplemental methods.

Results

A total of 1,004 individuals were assessed, 34% (345/1,004) of which were from LMICs, including Mexico (209), Peru (89), the Democratic Republic of Congo (35), Ghana (9),

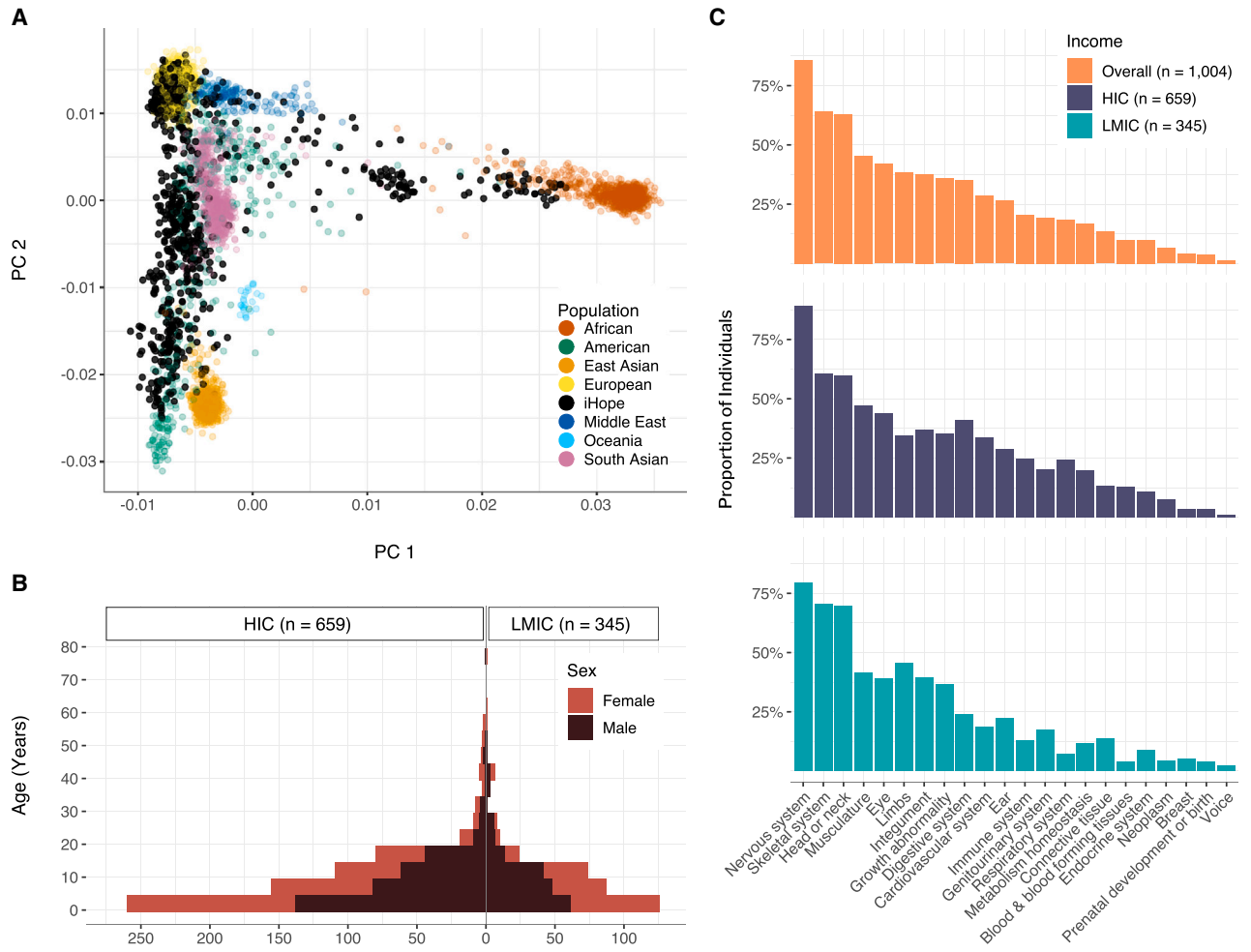

Figure 2. Demographics and phenotypic presentation of the iHope cohort

(A) Principal component analysis of the iHope cohort individuals (black) overlayed on seven human superpopulations derived from the 1000 Genomes, Human Genome Diversity, and Simons Genome Diversity datasets.

(B) Age and sex distributions stratified by high-income country (HIC) and low- and middle-income country (LMIC) sites. HIC age (y): mean 8.9, median 6.3, range 0 days–77.1 years; LMIC age (y): mean 9.6, median 6.6, range 26 days–77.9 years with two males of unknown age assigned the mean age for individuals from LMIC sites. HIC sex: male 350/659 (53.1%), female 309/659 (46.8%); LMIC sex: male 187/345 (54.2%), female 158/345 (45.7%). There are no statistically significant differences in age and sex distributions between the HIC and LMIC populations ($p = 0.36$ and $p = 0.69$).

(C) Summary distribution of top-level Human Phenotype Ontology terms nested beneath "Phenotypic abnormality" (HP:0000118) across the iHope cohort and stratified by HIC and LMIC.

Romania (1), Brazil (1), and India (1) (Figure 1). Individuals were from diverse ancestral backgrounds, with representation from each of seven superpopulations derived from the 1000 Genomes Project, Human Genome Diversity Project, and Simons Genome Diversity Project cohorts (Figures 2A, S2, and S3). A non-European superpopulation was the highest ancestral contributor in 51.8% (521) of the cohort. There was not a statistically significant difference in age distribution between individuals from LMIC sites and HIC sites (median age [y] LMIC 6.6 [range 26 days–77.9 years] vs. HIC 6.3 [range 0 days–77.1 years], point estimate of the difference between the HIC and LMIC populations 0.3, 95% CI 0.7–1.9, $p = 0.36$) (Figure 2B). Trio or higher-order family structures including both biological parents were available for the majority of families (713/1,004 [71.0%]; LMIC 228/345 [66.1%] vs. HIC 485/659 [73.6]), and for sex, the odds ratio male was 1.1 (95% CI 0.8–1.4, $p = 0.69$; LMIC 187/345 [54.2%] vs. HIC 350/659 [53.1%]) (Figure 2B).

Phenotypes were complex with nervous system, skeletal system, and head or neck the most frequently identified Human Phenotype Ontology root ancestor terms in both LMIC and HIC groups (Figure 2C). Digestive system (LMIC 24% vs. HIC 41%), respiratory system (LMIC 7% vs. HIC 24%), and cardiovascular system (LMIC 19% vs. HIC 34%) root ancestor terms had the largest proportional difference between LMIC and HIC groups. Individuals from HIC sites had an average of 2.3 more phenotypic terms submitted per individual (95% CI 1.5–3.1, $p < 0.001$) but computed phenotypic information content (an average of 6.4 IC bits per individual) showed no association with likelihood of a positive test result 95%

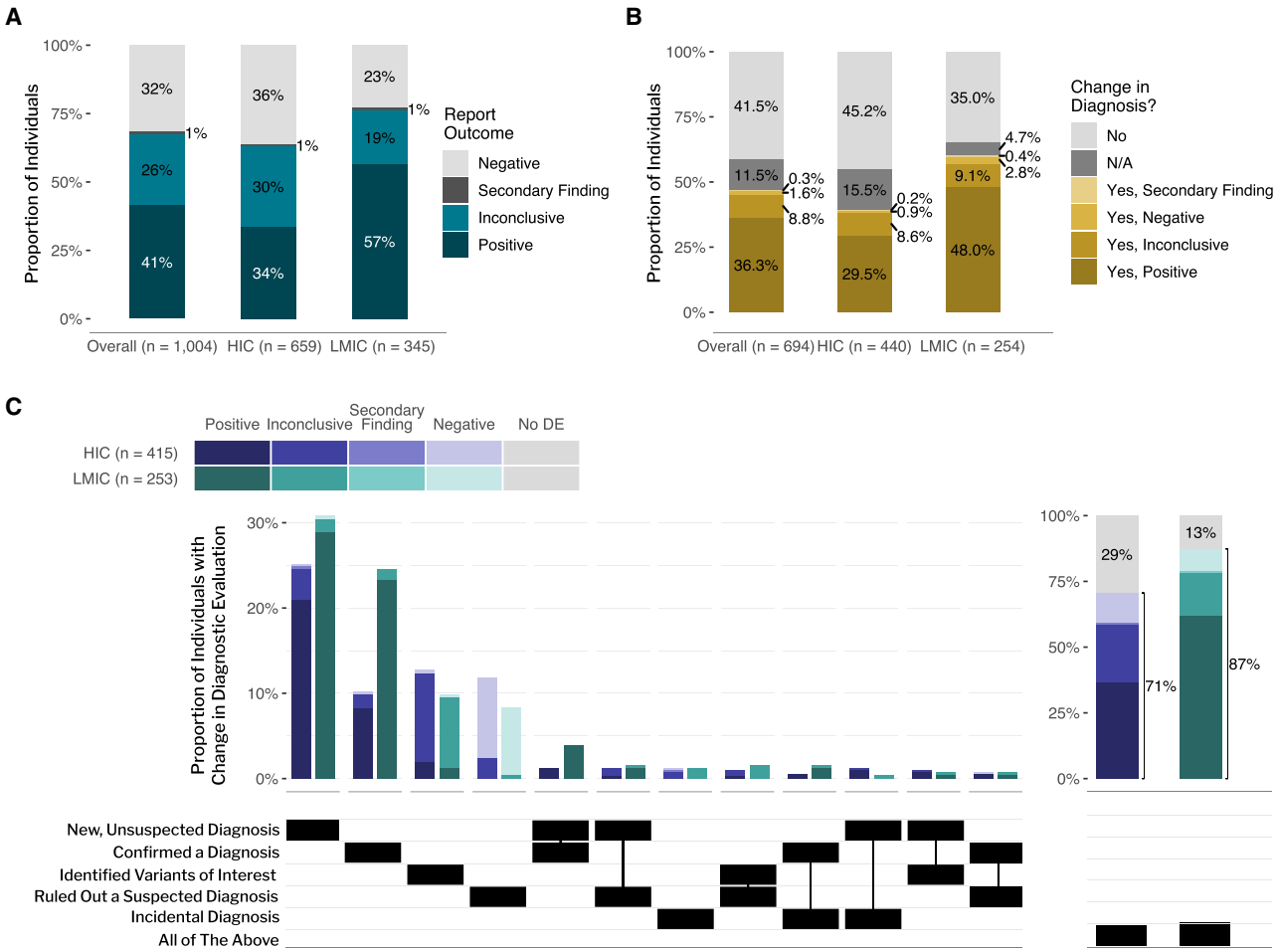

Figure 3. Diagnostic yield of cGS and its impact on clinical diagnosis and diagnostic evaluation

(A) Overall diagnostic yield of cGS stratified by test result category and by HIC and LMIC.

(B) Change in clinical diagnosis due to cGS grouped by HIC and LMIC sites. Survey response options included "yes," "no," and "not applicable." Survey responses endorsing a change in clinical diagnosis are stratified by test result category.

(C) The impact of cGS results on diagnostic evaluation. Response options are reflected in the text in the lower left, with black rectangles representing endorsement by the responding clinician. Multiple response options could be endorsed. The vertical black lines connecting black rectangles indicate a response combination. Bar plots above each set of responses indicate the proportion of individuals with a DE response combination stratified by test result category. Stacked bars to the far right reflect the combined total responses supportive of an impact on diagnostic evaluation.

CI 1.7–11.6, $p = 0.17$) (Figure S4 and supplemental methods).

The total diagnostic yield across the cohort was 41.4% (416/1,004), with individuals from LMIC sites 1.7 times more likely to receive a positive test result compared to individuals from HIC sites (LMIC 56.5% [195/345] vs. HIC 33.5% [221/659], OR 2.6, 95% CI 1.9–3.4, $p < 0.0001$) (Figure 3A). An additional 26.1% of individuals received inconclusive test results, which were more frequent in HIC (195/659, 29.5%) compared to LMIC (67/354, 19.4%) individuals (Figure 3A). Although individuals from HIC sites were 2.1 times more likely than individuals from LMIC sites to have had at least one prior genetic test (OR 6.6, 95% CI 4.9–8.8, $p < 0.0001$), there was no observed difference in the likelihood of a positive test result based on prior genetic testing within either the HIC or LMIC cohorts (Figure S5). Trio or higher-order family structures including the affected individual and both unaffected parents were more frequent in families from HIC sites (LMIC 228/345 [66.1%] vs. HIC 485/659 [73.6]) and so did not explain the higher diagnostic yield among LMIC sites.

Reported variants (1,033) spanned the mutational spectrum, including SNVs in the nuclear genome (714), small indels (130), CNVs (165), STRs (10), mitochondrial SNVs (9), uniparental disomy (3), and spinal muscular atrophy detected by bi-allelic absence of the *SMN1* c.840C allele (2) (supplemental methods, Figure S6). Individuals from LMIC sites had a greater proportion of copy number variants reported (LMIC 84/377 [22.3%] vs. HIC 81/650 [12.5%]), which also spanned a larger size range, compared to individuals from HIC sites (Figure S7).

Clinical GS impact surveys were completed by a clinical provider for 694 individuals (69.1% [694/1,004] of the total cohort) (Figure 1). Survey response rates were

comparable between LMIC and HIC sites (LMIC 254/297 [85.5%] vs. HIC 440/521 [84.5%]) and were highest for individuals with positive test results (LMIC 91.3% [157/172] vs. HIC 92.0% [162/176]).

To control for the elevated LMIC diagnostic yield and the potential influence of LMIC or HIC site designation on DE and COM, which as reflected in Figure S1 is mediated by test result category, comparative analyses of GS test impact were stratified by test outcome. Clinical GS results impacted DE in a total of 76.9% (514/668) of individuals and was more common in those from LMIC sites regardless of test result category (LMIC 87.4% [221/253] vs. HIC 70.1% [293/415], OR 2.2, 95% CI 1.3–3.6, $p < 0.0001$) (Figures 3C and S8B; Table S5). Overall, individuals with a positive test result were 64.2 times more likely to have DE impacted (95% CI 32.5–202.8, $p < 0.0001$) (Figure 3C). Clinical GS findings led to a change in the clinical diagnosis in a total of 46.9% (326/694) of individuals and was significantly more likely to occur in individuals from LMIC sites compared to HIC sites (LMIC 60.2% [153/254] vs. HIC 39.3% [173/440], OR 1.4, 95% CI 0.9–2.1, $p = 0.04$) (Figures 3B and S8A). Positive test results corresponded to the highest rates of change to the clinical diagnosis and were comparable between HIC and LMIC sites (HIC 79.8% [130/163] vs. LMIC 77.7% [122/157], OR 0.9, CI 95% 0.5–1.5, $p = 0.50$).

Clinical GS resulted in a change in management in 41.1% (285/694) of individuals, inclusive of specialty referrals, imaging and testing, therapeutic interventions, and palliative care (Figures 4A–4C and S9). When comparing across GNI site designations, individuals with a positive test result from LMIC sites were equally likely to experience a COM compared to individuals with a positive test result from HIC sites (OR 0.9, 95% CI 0.5–1.6, $p = 0.49$), and no statistically significant differences were observed among inconclusive or negative test results (Table S5). When genetic counseling and avoidance of additional testing were also considered, a total of 69.2% of individuals experienced COM (LMIC 77.9% [198/254] vs. HIC 64.1% [282/440]) (Figure 4A).

Overall, genetic counseling was the most frequently endorsed change in management category (62.6% [377/602]), followed by referrals, imaging and testing (42.9% [265/617]), avoidance of additional testing (29.6% [198/668]), therapeutics (12.3% [71/575]), and palliative care (3.3% [23/694]) (Figures 4 and S10). Therapeutic COM was endorsed in 72 individuals and was comparable between HIC and LMIC sites (HIC 11.9% [42/353] vs. LMIC 13.5% [30/222], OR 0.9, 95% CI 0.5–1.5, $p = 0.62$) (Figures 4A and 4C).

Among LMIC individuals, six exemplar cGS diagnoses with changes of management were noted (Table 1). For example, in a 22-year-old female with early-onset spastic paraparesis, lower limb hyperreflexia, and classic spastic gait, cGS identified a heterozygous, paternally inherited in-frame insertion in *GCH1* (MIM: 600225), classified as likely pathogenic for dystonia, DOPA-responsive (DRD [MIM: 128230]). This finding resulted in a change of the individual's clinical diagnosis from early-onset hereditary spastic paraparesis to DRD, and both she and her affected father, previously diagnosed with sporadic early-onset parkinsonism, responded to low doses of levodopa with almost complete control of symptoms.

Discussion

There is increasing evidence supporting the value of cGS as a first-line test in pediatric individuals with a suspected rare genetic disease, particularly in large cohorts drawn from single high-income geographies.[8,14] There has been little investigation, however, of the impact of cGS in individuals with reduced access to medical care. This is likely driven by three factors: (1) challenges deploying cGS to individuals with reduced resources, the majority of whom fall outside academic medical center referral areas; (2) difficulties maintaining individual and clinician engagement to enable assessment of COM after the administration of cGS; and (3) concern that the complexities of pediatric genetic disease, in combination with limited local resources, may introduce additional barriers to therapeutic access and therefore that cGS may not yield meaningful clinical benefit to individuals in resource-encumbered settings. The iHope program has directly addressed the first two challenges and enabled assessment of the third.

The analyses performed in this geographically diverse cohort focused on three elements of potential cGS impact—diagnostic yield, diagnostic evaluation (DE), and change of management (COM)—investigating the cohort as a whole and the differences between HIC and LMIC populations while controlling for possible confounders. The diagnostic yield of cGS across the cohort was comparable to similar studies in genetically and phenotypically diverse cohorts but was increased in individuals from LMIC sites compared to HIC sites.[6,7,13] This difference is likely explained by differential test utilization as individuals from HIC sites were 2.1 times more likely than individuals from LMIC sites to have had at least one prior genetic test, suggesting that cGS in individuals from LMIC sites was more often utilized as a first-line test, potentially in individuals with more severe phenotypic presentations. This hypothesis is supported by cGS detection of an increased number and size of copy number variants in individuals from LMIC sites, which likely would have been identified by chromosomal microarray or karyotype if otherwise available.

As reflected in Figure 2C, selection for cGS testing through the iHope program was consistent with genetic testing guidelines and prior investigations of the impact of exome and genome sequencing in individuals with suspected rare genetic disorders, inclusive of individuals with neurodevelopmental disorders, multiple congenital anomalies, and epilepsy.[8] However, to control for the elevated diagnostic yield in individuals from LMIC sites and the influence of site-specific selection variation on DE and COM

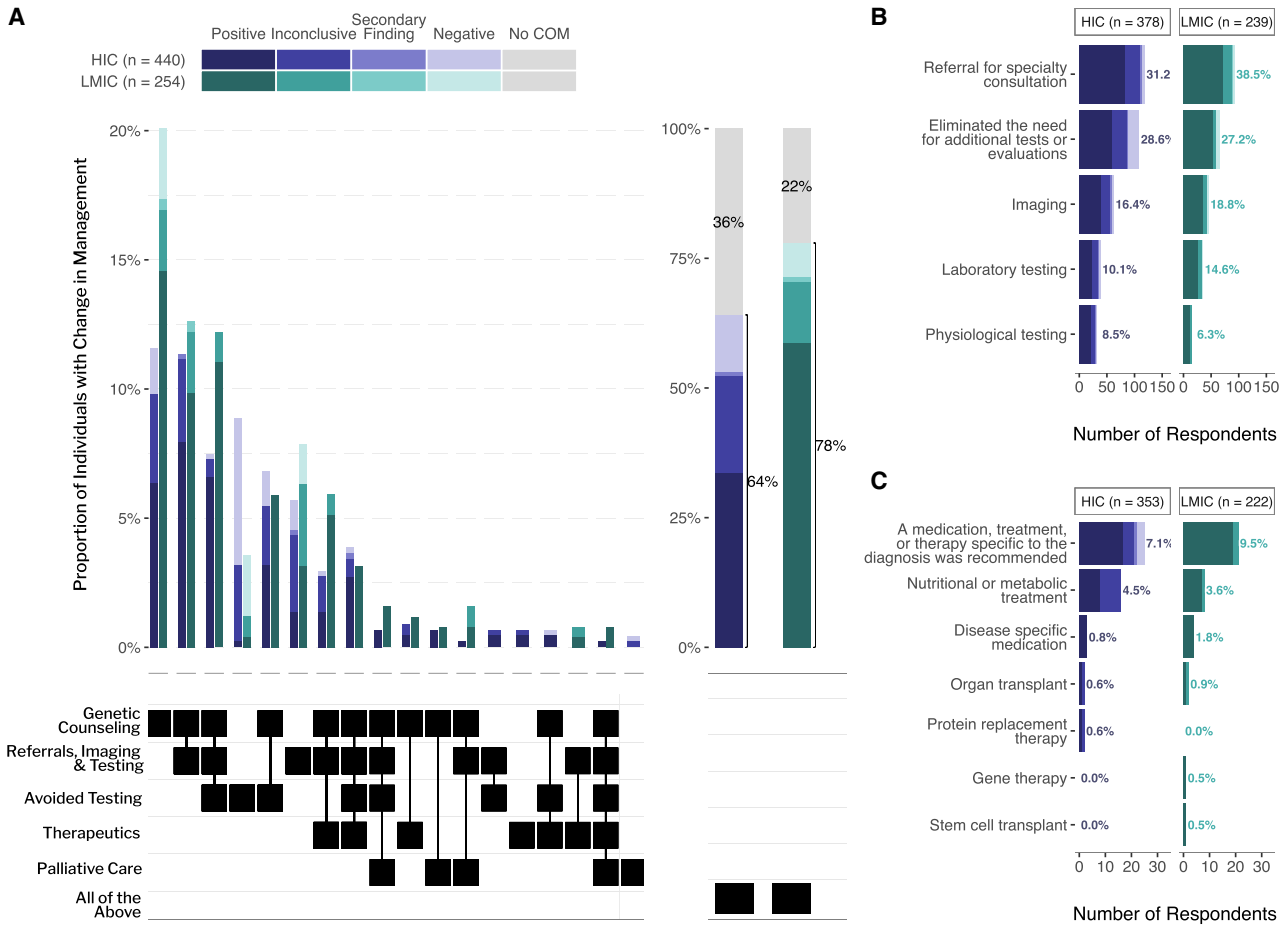

Figure 4. Changes of management associated with cGS testing results

(A) Change of management (COM) across the cohort and stratified by HIC and LMIC sites and test result category. Response options are reflected in the text in the lower left, with black squares representing endorsement of a COM category. Multiple response options could be endorsed. The vertical black lines connecting black rectangles reflect a combination of endorsed responses. Bar plots above each set of responses indicate the proportion of individuals with the indicated COM response combination stratified by test result category.

(B) Distribution of COM categorized to the referrals, imaging and testing COM category, stratified by HIC and LMIC sites and test result category.

(C) Distributions of COM categorized to the therapeutics COM category, stratified by HIC and LMIC sites and test result category.

(Figure S1), analyses were stratified by test result category. The impact of cGS on DE revealed a high proportion of individuals across the cohort with results that informed clinician assessment, with more individuals from LMIC sites experiencing a change in DE compared to individuals from HIC sites even when stratified by test result category. This may in part reflect implicit phenotypic heterogeneity given the diversity of rare genetic diseases, and differential prior testing and clinical specialist availability at LMIC sites. Differences in clinician levels of genetic education may offer an alternate or partial explanation, which is supported by increased endorsement of "change to clinical diagnosis" for individuals from LMIC sites with negative test outcomes compared to HIC (Figure S8B; Table S5). Assessment of COM revealed that up to 70% of individuals had a change in clinical care. When stratified by test result category, the overall likelihood of COM was comparable between the LMIC and HIC populations, suggesting that precision care in RGD populations is mediated by access to an etiological diagnosis. Indeed, as highlighted in Table 1, unanticipated changes in management can lead to marked improvements in quality of life for individuals in LMICs.

Recent studies have raised the possibility that access to cGS or other broad molecular tests such as exome sequencing supports diagnostic equity.[13,23] The data presented here further strengthens this position and suggests that for individuals with RGD, the highest likelihood of receiving precision care is tied to a precision diagnosis, irrespective of geographic location or resourcing of the local care team. Despite the high rate of COM in the LMIC population, lack of access to therapeutic interventions was reported, illustrating the need to bolster international funding for under-resourced clinical systems. For example, an individual from the Democratic Republic of the Congo with *THRA*-related congenital non-goitrous hypothyroidism (MIM: 614450) was referred to an endocrinologist for specialty evaluation and medication management but

Table 1. Notable Management Changes in Individuals from LMIC sites

Study ID; age group; sex	Phenotype	Clinical diagnosis prior to cGS	cGS finding & disorder	MIM #: gene; disorder	Change in management
00638; 6-18 years; male	early-onset generalized dystonia, hyperreflexia, knee pain, cramping, bilateral renal hydronephrosis	early-onset dystonia (G24.9)	het. 71 kb deletion including exon 1 of *GCH1*; unknown inheritance (PO); P; dopa-responsive dystonia (AD)	600225; 128230	low doses of levodopa/decarboxylase inhibitor
00800 >18 years female	early-onset spastic paraparesis with lower limb hyperreflexia, spastic gait, mild scoliosis, pes equinovarus	early-onset spastic paraplegia (G11.4)	het. paternally inherited in-frame insertion in *GCH1*; LP; dopa-responsive dystonia (AD)	600225; 128230	low doses of levodopa/decarboxylase inhibitor
00316 1–5 years female	muscle hypotonia, hyporeflexia, foot malposition, myopathic facies, normal early motor development but unable to walk independently	spinal muscular atrophy	absence of the *SMN1* c.840C allele consistent with absence of wild-type *SMN1* & predicted to result in spinal muscular atrophy	600354; 253300	Nusinersen
00818 6–18 years male	moderate intellectual disability, DD, short stature, epilepsy, obesity, aggressive behavior, poor attention & dysmorphic facial features	undiagnosed intellectual disability with obesity & short stature	het. *de novo* missense variant in *SLC2A1*; P; glucose transporter type I deficiency syndrome (AD)	138140; 606777	ketogenic diet with carnitine supplementation
00346 6–18 years male	progressive hypotonia, weakness & muscle atrophy especially pronounced in lower extremities & proximal limbs, elevated CK	undiagnosed muscular dystrophy	hemizygous, maternally inherited intronic variant in *DMD*; LP; dystrophinopathies (XL)	300377; 310200, 300376	cardiology referral, deflazacort recommended
00338 1–5 years male	epilepsy, heterotopia, NDD, allergic rhinitis, cerebral palsy, generalized weakness, uncoordinated walk, hypertrichosis, minor dysmorphic features, focal cortical dysplasia	undiagnosed developmental disorder	het. *de novo* missense variant in *GFAP*; P; Alexander disease (AD)	137780; 203450	recommended to follow neuroprotective measures such as avoiding long fasting periods, flickering lights & continuing care with a neuro-pediatrician

Y, years; (G.XX.X), ICD code provided by clinician; kb, kilobase; PO, proband-only analysis; AD, autosomal-dominant inheritance of the preceding disorder; XL, X-linked inheritance of preceding disorder; LP, likely pathogenic variant classification; P, pathogenic variant classification; DD, developmental delay; NDD, neurodevelopmental delay; het, heterozygous.

was lost to follow-up after the family indicated they could not afford the recommended changes in care. Unlike infectious diseases, for which there is an extensive philanthropic network to support surveillance, diagnosis, and treatment, individuals with suspected RGDs remain underrecognized and underserved. Multi-national public-private partnerships focused on the delivery of diagnostics and therapeutics in LMICs are necessary to reduce the morbidity and mortality associated with monogenetic disorders.

The iHope program encountered three implementation challenges regardless of geographic location: community trust, logistics, and local regulations. iHope was able to address community trust by: (1) providing a validated clinical genomic test deliverable in a reasonable turnaround time, (2) establishing relationships with local members of the clinical community, based on mutual respect, who were able to advocate on behalf of the program, and (3) reinforcing that individuals and clinicians were able to request access to their genomic data at any time. Logistical challenges were concentrated on the collection and shipment of blood or extracted DNA to the Illumina Clinical Services Laboratory. This was generally addressed on a case-by-case basis and by catering to local needs to batch samples or to ship them as collected. Regulations varied by site and continue to evolve, requiring ongoing conversations with each clinical site to ensure program compliance. iHope relied on the clinical sites to inform program administration when specific regulations needed to be addressed.

Limitations of this investigation include its duration and potential differences in barriers to obtaining access to the iHope program (which are likely to mimic inherent limitations in the availability and accessibility of molecular testing for individuals with RGD). Although survey response rates were comparable between LMIC and HIC sites and statistical analyses were employed that controlled for test outcome, cGS impact surveys were not returned for all individuals, which may introduce an additional unquantifiable bias. The clinical impact survey also changed over time, as detailed in Table S4, in response to clinical site feedback and developing recommendations.[24] Lastly, grouping sites into two broad categories, HIC and LMIC, offers only a "high-level" framework to assess cGS impact in LMICs and does not consider other important factors such as national healthcare models or other country-specific variables which were beyond the scope of this investigation.

Future studies investigating clinically indicated cGS test utilization in the context of clinician ordering behaviors, and detailed analyses of resource encumbrances, will refine our understanding of appropriate care and implementation pathways in LMICs. It is likely that genomic test cost will be a barrier to implementation in some geographies, and therefore additional studies to assess the cost-benefit of different testing regimes, inclusive of genomes, exomes, microarray, and panels, are needed to guide region-specific sustainable solutions that can be integrated into the local healthcare system.

Conclusions

In a diverse and global cohort of individuals with suspected rare monogenic diseases, cGS testing led to high rates of molecular diagnosis and changes to both diagnostic evaluation and clinical management. These findings suggest that equitable access to clinical genomic testing, in concert with additional investments in LMICs to support RGD clinical care, may reduce global healthcare inequities.

Data and code availability

The datasets and code supporting the current study have not been deposited in a public repository to ensure anonymity and privacy. De-identified outcomes data detailed in this manuscript can be used for further study. Clinical sequencing data will not be made available to external researchers given the privacy and protections inherent to a clinical sequencing test. Per routine laboratory practice, the Illumina Clinical Services Laboratory reported variants identified in the iHope population to ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/submitters/504895/. Data analysis code is available upon request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2024.05.006.

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Declaration of interests

E.T., E.C., K.R., J. Button, A.M., M.B., J.A., A.W., M.A., T.K., A.C., S.S.A., D.L.P., and R.J.T. were employees of and stockholders in Illumina, Inc. at the time of this investigation. V.R. is a stockholder in Illumina, Inc. J.O. is a stockholder in Illumina, Inc. and employee of C2N Diagnostics. J. Belmont and T.W. are stockholders in Illumina, Inc. and were compensated as research advisors through Genetics & Genomics Services Inc. C.S. was compensated as a consultant through Genetics & Genomics Services Inc. for statistical analysis. K.M. is an employee of Ambry Genetics.

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Web resources

GenBank, https://www.ncbi.nlm.nih.gov/genbank/
Genetic Alliance, https://geneticalliance.org/ihope-genetic-health
OMIM, https://www.omim.org/
World Bank Country and Lending Groups – World Bank Data Help Desk , https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups

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