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Clinical sites of the Undiagnosed Diseases Network: unique contributions to genomic medicine and science

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Purpose: The NIH Undiagnosed Diseases Network (UDN) evaluates participants with disorders that have defied diagnosis, applying personalized clinical and genomic evaluations and innovative research. The clinical sites of the UDN are essential to advancing the UDN mission; this study assesses their contributions relative to standard clinical practices.

Methods: We analyzed retrospective data from four UDN clinical sites, from July 2015 to September 2019, for diagnoses, new disease gene discoveries and the underlying investigative methods.

Results: Of 791 evaluated individuals, 231 received 240 diagnoses and 17 new disease–gene associations were recognized. Straightforward diagnoses on UDN exome and genome sequencing occurred in 35% (84/240). We considered these tractable in standard clinical practice, although genome sequencing is not yet widely available clinically. The majority (156/240, 65%) required additional UDN-driven investigations, including 90 diagnoses that occurred after prior nondiagnostic exome sequencing and 45 diagnoses (19%) that were nongenetic. The UDN-driven investigations included complementary/supplementary phenotyping, innovative analyses of genomic variants, and collaborative science for functional assays and animal modeling.

Conclusion: Investigations driven by the clinical sites identified diagnostic and research paradigms that surpass standard diagnostic processes. The new diagnoses, disease gene discoveries, and delineation of novel disorders represent a model for genomic medicine and science.

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INTRODUCTION

The Undiagnosed Diseases Network (UDN) is a research consortium supported by the National Institutes of Health (NIH) Common Fund to diagnose participants with refractory medical conditions and conduct collaborative research on the etiology and pathophysiology of undiagnosed diseases.[1–4] Accepted participants undergo personalized clinical and research evaluations at 1 of 12 clinical sites. Since 70–80% of undiagnosed diseases are due to rare and ultrarare genetic disorders (affecting fewer than 620 patients and fewer than 20 patients per million of the population, respectively),[5,6] sequencing plays a central role in the UDN, with exome sequencing (ES) and genome sequencing (GS) performed through collaborations among the clinical sites and the sequencing core laboratories. Emerging laboratory methods, such as RNA sequencing (RNASeq) analyses for resolving the significance of genetic variants, occur at the clinical sites. Additional network resources including model organism screening centers (MOSCs), a metabolomics core laboratory, a coordinating center, and a biorepository enhance the network's research mission. The UDN diagnostic rate is ~30%,[3] noteworthy in view of the extensive prior diagnostic

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failures, including nondiagnostic ES. This enhanced diagnostic yield in these extremely difficult cases is due to a combination of methods, including customized clinical evaluations with detailed phenotyping, innovative genomic analysis, and, importantly, close collaborations within the network and outside, including data sharing.[3] These activities allow the UDN to diagnose refractory cases, overcoming existing constraints of clinical practice and of disease-, technology-, or body system–limited clinical research programs.

ES is increasingly available through other research studies and in clinical practice, with a diagnostic yield of 25–40% in ES naïve probands.[7–10] When third party or public payers cover costs, general genetics and exome clinics can utilize ES in their diagnostic protocol.[11–15] However, several factors complicate the clinical application of ES.[16–19] First, most participants who undergo ES obtain a nondiagnostic result, either with no variants, or variants of uncertain significance (VUS) that cannot be resolved further through clinical means. Pursuit of candidate genes often requires further research-level verification. Case-matching resources such as GeneMatcher[20] are powerful, but often do not produce successful collaborations. Variants of uncertain significance in known disease genes can remain unresolved, despite clinical follow-up studies in >50%.[10,21] Subsequent reanalysis of nondiagnostic ES data by clinical laboratories can yield diagnoses in up to 15% of cases.[22–27] However, clinicians have few further options if this reanalysis is also nondiagnostic. Second, in >10% of cases, differences in the interpretation of ES between the ordering clinician and testing laboratory occur, and may be difficult to resolve.[21] Third, clinical laboratories may not report variants in known disease-associated genes if there is phenotypic discordance, or variants in genes not currently associated with disease, leading to missed opportunities for clinicians to solve cases.[28] Finally, time and reimbursement are significant barriers to pursuing all the above activities. Clinical ES utilization is rising,[29–31] but pre- and post-ES activities are labor intensive, requiring time outside the reimbursable face-to-face clinical encounters.[29,32,33]

The UDN has pioneered and optimized diagnostic and research strategies to overcome these constraints. Geographic and financial barriers are minimized, with participants being accepted solely on the clinical manifestations, thereby providing equitable enrollment and evaluations. It is unique among genomic research networks in adopting the N-of-1 paradigm, rather than a cohort approach, enabling personalized in-person phenotyping and genomic evaluations beyond what is available in a clinical setting, or in studies that gather historical records and perform genomic evaluations. Collaborative science within the network facilitates disease gene discovery.

Genomic data analyses in the UDN have distinctive elements. First, dual analysis of UDN-generated sequencing data is available at the sequencing core laboratory and the individual clinical sites. Second, reanalysis of raw data from pre-UDN nondiagnostic ES and GS occurs at the clinical sites. The UDN clinical sites' research analytical pipelines and other adjunct methods can identify variants that a commercial laboratory might not report, resulting in a resolution rate of 40% in the ES nondiagnostic cases, as previously reported.[28] Third, new and innovative analysis techniques are periodically applied to unsolved cases, since no such case is considered "closed." Despite the inherent clinical and etiological heterogeneity of the participants, the network has been successful in making diagnoses, discovering disease-causing genes, and expanding rare disease phenotypes using data sharing and collaborative efforts.[3,28] The UDN paradigm goes well beyond what is currently possible in a busy and time-constrained clinical diagnostic setting. We illustrate the value of this with an in-depth analysis of data from four UDN clinical sites.

MATERIALS AND METHODS

Ethics statement

This retrospective study was conducted under the UDN protocol approved by the central institutional review board at the National Human Genome Research Institute, with referral, review, and evaluation processes previously described.[3] Informed consent was obtained from all subjects.

Participating sites

Invitations for participation were extended to the seven clinical sites that had been part of the UDN since its inception in 2014. Four clinical sites participated, including Duke/Columbia University Medical Centers, the NIH Undiagnosed Diseases Program, Stanford Medicine, and Vanderbilt University Medical Center. Application and evaluation processes performed at these four sites are representative of all clinical sites, described in the UDN manual of operations and published previously.[3] The data collection period was July 2015 through September 2019. The full cohort included all applicants to these clinical sites and, among these, a smaller cohort of individuals who were accepted, evaluated, and received a diagnosis. Demographic information as well as details of the diagnoses, internal collaborations, and geographic location of the participants, were downloaded from the UDN Gateway.[34] The clinical sites provided details on UDN-driven investigations for all diagnoses. A subset of the diagnoses and disease genes (September 2015–May 2017) was published previously.[3] Management changes due to diagnoses have been reported.[4] and are not included in this paper. Quantitative analyses were conducted using SPSS 26.0.

UDN-driven investigations

The clinical sites collaboratively initiated and completed several research processes to increase the rate of diagnoses. All applicants underwent a medical record review that identified steps for a personalized evaluation, including phenotyping, sequencing, or other laboratory tests as follows:

1. **Complementation/supplementation of prior clinical data:** Phenotypic gaps were filled and new diagnostic clues were sought through temporally concentrated specialty evaluations, re-examination of prior studies (including

imaging, pathology slides, etc.), and phenotype-driven imaging, procedures, and genetic/nongenetic laboratory studies. If UDN genomic results were already available at the time of the clinical evaluation, the phenotyping was further customized, as indicated.

2. **Innovative analysis of genomic data and collaborative investigations to advance genomic science**: When feasible, pre-existing raw sequence data (FASTQ/BAM) from prior nondiagnostic ES were reanalyzed through research pipelines at the clinical sites.[28,35] A nondiagnostic ES was operationalized as one in which there were no variants, or a single heterozygous variant was found in genes for autosomal recessive conditions, or VUSs in novel candidate or known disease-associated genes that could not be resolved further. For others, new ES or GS was performed at the UDN sequencing core laboratory, with dual analysis of the data by the core and the clinical sites. Emerging variants were manually curated at the clinical sites (see Supplementary materials). Functional assays such as reverse transcription polymerase chain reaction (RT-PCR) were performed when indicated. Variants of interest were further evaluated using internal data sharing, case matching, animal modeling, and RNASeq. External data sharing for case matching and collaborations occurred through PhenomeCentral, GeneMatcher,[24] myGene2,[36] and through individualized UDN participant webpages.[36]

Classification of UDN diagnoses

In accordance with the UDN diagnosis-coding tool,[3] the diagnoses made after UDN evaluation were classified as (1) clinical diagnoses, (2) diagnoses due to phenotype-directed testing, (3) diagnoses stemming from ES/GS, or (4) diagnoses on nonsequencing, genome-wide diagnostic assay (chromosomal microarray [CMA] (footnote in Table 2 defines the diagnosis classification). New disease gene discoveries were included in these diagnoses.

Comparisons to clinical genetics practice

To assess if ES in the UDN alone with phenotype integration was enough to achieve a diagnosis (analogous to clinical practice), the clinical sites were asked to categorize each diagnosis as (1) straightforward or (2) requiring additional UDN-driven investigations detailed above, and to describe those. Straightforward diagnoses on ES were defined as those in which the UDN ES or GS detected compelling variant(s) that matched the phenotype, even if that phenotype was obtained by the UDN-driven deep complementary phenotyping. These diagnoses were considered achievable in current standard clinical practice. Straightforward diagnoses on GS were also included as not requiring additional UDN investigations, because GS will soon become increasingly available in clinical practice.

For further comparisons to clinical practice, the genetics clinics at Duke, Stanford, and Vanderbilt provided information on distances traveled by non-UDN patients to their general genetics clinics and results from these genetics clinics' ES reanalysis through commercial laboratories, when ES had been nondiagnostic. The NIH clinical site does not have a comparable clinical genetics practice and so was not included in these comparisons.

Table 1 Demographics, presenting clinical manifestations and pre-UDN ES status of the 231 diagnosed individuals (who had a total of 240 diagnoses).

Variable		Value (%)
Median age	Pediatric ($n = 155$)	6 years
	Adult ($n = 76$)	34.5 years
Gender	Female	131 (57%)
	Male	100 (43%)
Race	White	179 (78%)
	Asian	18 (8%)
	Black	13 (5%)
	Other	21 (9%)
Ethnicity	Hispanic	26 (11%)
	Non-Hispanic	168 (73%)
	Unknown	37 (16%)
Presenting symptoms category[a]	Neurologic	130 (56%)
	Multiple congenital anomalies	18 (8%)
	Musculoskeletal	17 (7%)
	Other (18 systems)	66 (29%)
Pre-UDN ES	Prior ES performed was nondiagnostic[b]	90 (39%)
	No prior ES	141 (61%)

ES exome sequencing, *UDN* Undiagnosed Diseases Network.
[a]Reported by clinical site at application review.
[b]Prior nondiagnostic ES defined as one with either (1) no variants, (2) heterozygous variants in autosomal recessive disease genes, or (3) variants of uncertain significance in a known disease gene or in a novel candidate gene, which could not be resolved further.

RESULTS

Demographics

Across the four clinical sites, 2490 applications were received and 964 individuals (39%) were accepted; clinical and genomic evaluations were complete on 791 participants (remainder 173 are undergoing evaluations). Of the 791 evaluated, 231 (29%) received 240 diagnoses; seven received two diagnoses and one received three. The median time to diagnosis (time from start of in-person clinical evaluation to results disclosure) was 185 days (0–1399). Of the 231 diagnosed individuals, 90 had a pre-UDN nondiagnostic ES (Table 1). Details of each diagnosis are in Table S1.

UDN-driven investigations

1. Complementation and supplementation of prior clinical data: Detailed and iterative phenotyping led to 16 clinical diagnoses, mainly nongenetic (case examples 1 and 2 in Table 2, Fig. 1, and Table S1). Phenotyping also identified differential diagnostic possibilities that were confirmed by

Table 2 Classification of diagnoses ($n = 240$) and case examples describing UDN-driven investigations leading to diagnoses.

Classification of diagnosis	Example case description	Diagnosis	UDN-driven investigations to establish diagnosis
Clinical diagnoses[a] ($n = 16$, 7%)	Case 1: 22-year-old male with episodic thrombocytopenia, lymphopenia, neutropenia, recurrent fevers	Autoimmune thrombocytopenic purpura	**Complementation/supplementation of prior clinical data (aggregate evidence led to diagnosis):** B-cell flow cytometry and Fas-mediated apoptosis normal. Intermittent antiplatelet antibodies. Recurrent severe episodes of thrombocytopenia, (neutropenia possibly secondary to treatment), responded to anti-CD20 therapy but complicated by serum sickness, ultimately stabilized after bone marrow transplant on NIH protocol treating refractory autoimmune disease. This is a nongenetic diagnosis.
Diagnoses due to phenotype-directed testing[b] ($n = 29$, 12%)			
Nongenetic laboratory testing ($n = 12$)	Case 2: 60-year-old male with periodic fevers, monthly episodes lasting 7–10 days	Waldenstrom macroglobulinemia	**Complementation/supplementation of prior clinical data (aggregate evidence led to diagnosis):** IgM elevation mild but bone marrow aspirate flow cytometry showed 1% clonal population of kappa-restricted plasmacytoid CD38/CD138+ cells. This is a nongenetic diagnosis.
	Case 3: 26-year-old female with progressive limb girdle weakness	Anti-HMGCR myopathy	**Complementation/supplementation of prior clinical data (neurology evaluation) and subsequent targeted testing:** Intermittent weakness was consistent with autoimmune myopathy: anti-HMGCR and anti-MDA5 antibodies identified on myositis panel (not included in myositis panel prior to UDN). This is a nongenetic diagnosis.
	Case 4: 29-year-old male with acquired transfusion-dependent dyserythropoietic anemia	Autoimmune dyserythropoietic anemia	**Complementation/supplementation of prior clinical data (bone marrow aspirate) and subsequent targeted testing:** Bone marrow aspirate pathology led to molecular study: increased population of gamma-delta T cells likely mediating cytotoxic immune response against erythroid precursors. Responded to cyclosporine. This is a nongenetic diagnosis.
Single-gene testing ($n = 17$)	Case 5: 4-year-old female with neurodevelopmental regression and nondiagnostic pre-UDN ES	*PLA2G6*-associated neurodegeneration with brain iron accumulation 2B (MIM 256600)	**Complementation/supplementation of prior clinical data (neurology evaluation) and subsequent targeted testing:** Neurological phenotype consistent with NBIA: MLPA for *PLA2G6* revealed novel homozygous deletion encompassing noncoding exon 1, with RT-PCR demonstrating absent PLA2G6 expression.
	Case 6: 39-year-old male with cognitive decline, personality changes, cerebral volume loss, and nondiagnostic pre-UDN ES	Frontotemporal dementia (MIM 105550)	**Complementation/supplementation of prior clinical data (consultation with neurology expert at another UDN clinical site) and subsequent targeted testing:** Progressive phenotype was consistent with *C9orf72* repeat expansion disease; testing showed >44 repeats, not detectable on ES.
Diagnoses stemming from ES/GS and downstream analyses[c] ($n = 190$, 79%)			
Exome sequencing ($n = 116$)	Case 7: 6-month-old male with severe congenital hypotonia and fine tremor and nondiagnostic pre-UDN ES	Congenital myopathy with tremor (MIM 618524), due to de novo variant in *MYBPC1* NM_002465.3:c.776T>C (p.Leu259Pro)	**Innovative analysis of genomic data (reanalysis of pre-UDN nondiagnostic ES) and collaborations outside network (case matching and functional assays):** Detected on UDN reanalysis of raw ES data. Variant was not reported on pre-UDN ES due to perceived poor phenotypic fit. Three other similarly affected cases identified, leading to new disease association for *MYBPC1*.
	Case 8: 20-month-old male with infantile spasms, microcephaly, lamellar cataracts, developmental delays and nondiagnostic pre-UDN ES	Neurodevelopmental disorder with epilepsy, cataracts, feeding difficulties and delayed brain myelination (MIM 617393), due to de novo variant in *NACC1* NM_052876.3:c.892C>T (p.Arg298Trp)	**Innovative analysis of genomic data (reanalysis of pre-UDN sequences) and collaborations within and outside network (case matching):** Reanalysis of pre-UDN ES data prioritized a de novo variant in *NACC1*. Additional individuals identified through GeneMatcher and the UDN patient webpage, leading to new disease gene identification.

Table 2 continued

Classification of diagnosis	Example case description	Diagnosis	UDN-driven investigations to establish diagnosis
Genome sequencing ($n = 74$)	Case 9: 10-year-old female with congenital neuropathy, muscle weakness, calf atrophy, and abnormal gait, and nondiagnostic pre-UDN ES	Charcot–Marie–Tooth disease, axonal, type 2S (MIM 616155), caused by biallelic variants in *IGHMBP2* NM_002180.2:c.1730T>C (p.Leu577Pro) and c.1235+894C>A	**Innovative analysis of genomic data (dual analysis of UDN sequence data by clinical site) and functional assay at clinical site** Maternal missense pathogenic variant reported on pre-UDN ES. Clinical site reanalysis of UDN GS data revealed a paternally inherited cryptic intronic splice site acceptor. Direct sequencing of *IGHMBP2* cDNA with and without a NMD inhibitor confirmed inclusion of a mini pseudoexon in mRNA. The abnormal transcript was subject to NMD.
	Case 10: 31-year-old female with adult-onset, slowly progressive distal asymmetric myopathy with scapular winging, mild facial weakness and nondiagnostic pre-UDN ES	Multisystem proteinopathy 3 (MIM 615424), caused by a de novo heterozygous 500 base pair deletion encompassing exon 10 of *HNRNPA1* NM_031157.3:c.1063+15_*5-68del	**Innovative analysis of genomic data (dual analysis of UDN sequence data by clinical site + RNASeq) and collaborations within network (between the clinical site and the sequencing core)** Collaborative analysis of UDN GS data by the clinical site and sequencing core identified the deletion which was confirmed by qPCR. RNASeq on patient fibroblasts confirmed that the aberrant transcript escapes NMD and results in truncation of the M9 protein domain.
	Case 11: 6-year-old female with macrocephaly, developmental delay and dysmorphic features, and nondiagnostic ES	Kleefstra syndrome type 2, caused by a heterozygous 127-kb deletion of 7q36.1 involving exons 8–55 of *KMT2C*	**Innovative analysis of genomic data (dual analysis of UDN sequence data and manual curation by clinical site):** Not reported as diagnostic due to size and presence of deletions in normal individuals; however, manual curation of *KMT2C* isoforms by the clinical site revealed that this distal deletion occurred in the isoform most expressed in brain tissue, whereas the deletions in healthy individuals in DGV were proximal and not in the brain expressed isoform.
Diagnoses on nonsequencing, genome-wide diagnostic assay: CMA[d] ($n = 5$, 2%)	Case 12: 10-year-old female with severe developmental delay, absent speech, joint laxity, tethered cord, and nondiagnostic pre-UDN ES	Wieacker–Wolff syndrome (MIM 314580), due to a de novo heterozygous 95-kb deletion involving exon 1 of *ZC4H2*	**Innovative analysis of genomic data (CMA performed on more advanced platform):** Deletion was detected on a high density CMA. Pre-UDN SNP-based CMA did not detect the deletion, likely due to being below the level of resolution. Reason for nondetection in UDN GS unclear.

cDNA complementary DNA, *CMA* chromosomal microarray, *DGV* Database of Genomic Variants, *ES* exome sequencing, *GS* genome sequencing, *MLPA* multiplex ligation-dependent probe amplification, *mRNA* messenger RNA, *NBIA* neurodegeneration with brain iron accumulation, *NIH* National Institutes of Health, *NMD* nonsense-mediated decay, *qPCR* quantitative polymerase chain reaction, *RT-PCR* reverse transcription polymerase chain reaction, *SNP* single-nucleotide polymorphism, *UDN* Undiagnosed Diseases Network.

[a]Clinical diagnoses defined as diagnoses made on clinical grounds, including aggregate assessment of nonspecific test results; also conferred when clinical diagnostic criteria were met, or pathognomonic signs were present.

[b]Diagnoses due to phenotype-directed testing: When the clinical manifestations were suggestive of a disorder or group of disorders, further targeted genetic or nongenetic laboratory tests were performed or reviewed and led to diagnoses. These were further classified into "nongenetic laboratory testing" and "single-gene testing."

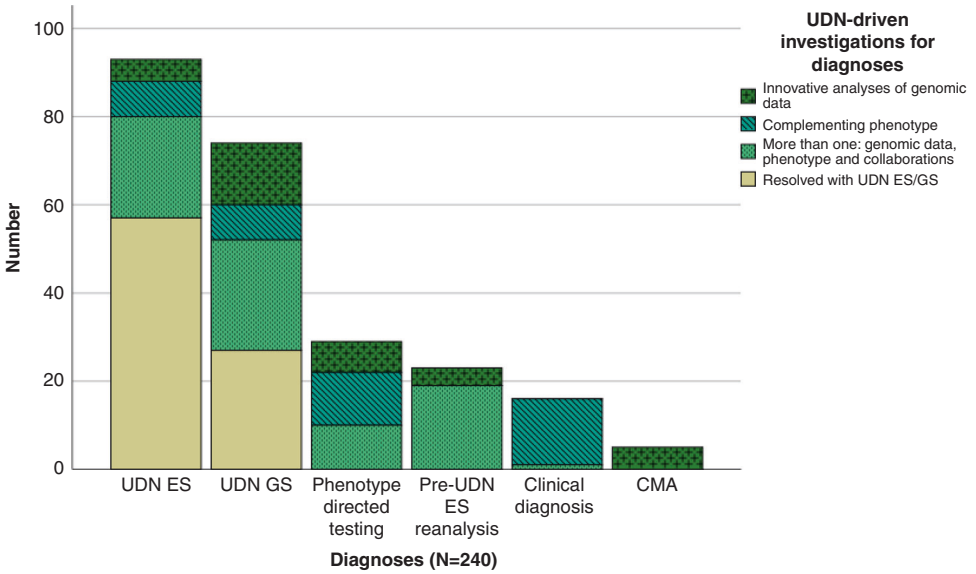

Fig. 1 Details of the 240 diagnoses. The beige portions of the bars indicate diagnoses that were made in a straightforward manner from exome/genome sequencing (ES/GS) that was performed by the Undiagnosed Diseases Network (UDN) sequencing core with integration of the phenotype by the UDN clinical sites. The 57 diagnoses (24%) that were due to UDN ES and 27 diagnoses (11%) that were due to UDN GS are similar to what could be accomplished in a regular genetics clinic. The green portions indicate diagnoses that were made with additional UDN-driven investigations that are difficult to accomplish in regular clinical settings. In aggregate the majority of diagnoses ($n = 156$ of 240, 65%) occurred due to the additional and most often multiple UDN-driven investigations, initiated at the clinical sites. *CMA* chromosomal microarray.

subsequent specific testing, producing a genetic or nongenetic diagnosis in 29 individuals (case examples 3–6 in Table 2, Fig. 1, and Table S1). Cumulatively, the phenotyping led to 45 of 240 (19%) diagnoses.

2. Innovative analysis of genomic data, nonsequencing genomic approaches, and collaborative investigations to advance genomic science: 190 of 240 diagnoses (79%) stemmed from reanalysis of prior nondiagnostic ES and new ES or GS performed in the UDN (Tables 2 and 3, S1). Broadly, ES led to 116 diagnoses and GS to 74 diagnoses. The UDN-driven genomic analyses were as follows.

Reanalysis of pre-UDN nondiagnostic ES data

Remarkably, of the 231 diagnosed individuals, 90 (39%) had a nondiagnostic ES prior to UDN enrollment. Reanalysis of pre-UDN sequence data was completed for 53 individuals and resulted in 23 diagnoses, with a 43% diagnostic rate with reanalysis (Table 3, case examples 7 and 8 in Table 2).

Other methods to resolve prior ES nondiagnostic cases. Among the remaining 67 (of 90) prior nondiagnostic ES, GS resolved 41 (45%) and repeat ES in the UDN (due to prior outdated ES) resolved another 8 (8%). The remainder of the ES nondiagnostic cases were resolved without genomic sequencing, i.e., via clinical diagnosis in 4 (4%), diagnosis on phenotype-directed testing in 11 (12%), and diagnosis on CMA in 3 (3%).

Comparison of the types of diagnoses in the ES nondiagnostic cases ($n = 90$) to the 150 diagnoses in the ES naïve cases showed no significant difference in the types of diagnoses (clinical diagnoses on phenotype-directed tests, genomic and CMA diagnoses) (Fig. S1). Regardless of whether ES had been performed previously, a personalized approach resulted in similar distribution of types of diagnoses, and in many of these reflexive GS following nondiagnostic ES would not have solved them.

Dual analysis of UDN-generated ES/GS data

Concurrent dual analysis of sequence data through the sequencing core pipeline and the clinical sites' research pipelines resulted in a larger number of candidate variants, as expected. These were manually curated at the clinical sites and reiteratively integrated with the phenotype, for prioritization as disease-associated or as novel genes (Table 3 and Supplementary materials). These clinical site investigations resulted in seven additional ES diagnoses and nine additional GS diagnoses beyond those issued in the report by the UDN sequencing core laboratory (case examples 9–11 in Tables 2, 3). Overall, 39 of the 190 (21%) genomic diagnoses were due to the clinical sites' analyses of pre-UDN and UDN-generated sequence data (Table 3). It is to be noted that straightforward ES ($n = 57$) and GS ($n = 27$) diagnoses were attributed to the sequencing core laboratory, even when the dual clinical site analysis detected these. The details of these straightforward diagnoses are below in "Comparison of the UDN to genetics clinics."

CMA diagnoses

Five diagnoses resulted from CMA studies, either because a prior CMA had never been performed or because a higher-resolution CMA was employed using the N-of-1 approach.

Table 3 Diagnoses made by ES or GS (190 of 240 diagnoses).

Sequencing and other efforts	Diagnoses on ES = 116			Diagnoses on GS = 74		Totals
	Pre-UDN ES data reanalysis at clinical sites	ES through UDN sequencing core	Clinical site dual analysis of UDN ES data	GS through UDN sequencing core	Clinical site dual analysis of UDN GS data	
Straightforward ES/GS diagnoses	0	57[a]	n/a[b]	27[a]	n/a[b]	84 (44%)
UDN investigations beyond ES/GS required for diagnosis[c]	23[d]	29	7[d]	38	9[d]	106 (56%)
Totals	**23**	**86**	**7**	**65**	**9**	**190**

ES exome sequencing, *GS* genome sequencing, *UDN*Undiagnosed Diseases Network.
[a]Diagnoses that were made on ES or GS through the UDN in a straightforward manner, by reconciling the ES or GS results with the phenotype, similar to a clinical genetics setting. The remainder ($n = 106$) needed UDN-driven investigations.
[b]When the diagnosis was identified by both the UDN sequencing core laboratory and on clinical site analysis, attribution was given to the UDN sequencing core.
[c]Details of additional investigations may be found in Table S1.
[d]Diagnoses ($n = 39$, 21%) solely attributed to UDN clinical sites' innovative research analyses of the ES and GS data.

These diagnoses demonstrate the utility of CMA in specific instances, e.g., case example 12 in Table 2.

RNASeq

Collaborations among UDN sites generated RNASeq data for 45 individuals in the diagnosed cohort and these contributed to diagnoses in 7 of 45 (16%). Example findings included allelic imbalance, evidence of functional impact of noncoding variants such as splicing, and evidence of nonsense-mediated decay (case examples 9 and 10 in Table 2).

New disease gene identification with collaborative science

Seventeen new gene–disease associations were included among the 190 sequencing diagnoses (Table S2). These discoveries arose through multiple avenues (case example 8 in Tables 2, S1). Collaborations with the UDN MOSC contributed to 8 of the 17 novel disease gene discoveries. Other internal collaborations included partnerships with the UDN Metabolomics Core and disease or technological experts at UDN sites (Table 2). In aggregate, internal data sharing and collaboration occurred for 131/240 (55%) diagnoses. External collaborations occurred for 64/240 (27%) diagnoses, with 34/64 (53%) contributing to the diagnosis.

Comparison of the UDN to genetics clinics

Table 4 describes UDN processes relative to standard clinical practices. Straightforward diagnoses on UDN ES and GS provided 84 diagnoses (35% of all 240), due to compelling sequence variation and congruence with the presenting phenotype (Fig. 1, Table 3). The remaining 156 diagnoses (65%) that were not straightforward required additional UDN-driven investigations that are difficult in clinical practice, with many requiring more than one such investigation (Fig. 1, Tables 2, and S1).

The 84 straightforward ES ($n = 57$) and GS ($n = 27$) diagnoses could be comparable to what could occur in standard clinical practice. However, 4 of the 57 straightforward ES diagnoses had been missed with a pre-UDN ES, due to interim new disease gene reports ($n = 1$), and analytical pipeline differences ($n = 3$). Of the 27 straightforward GS diagnoses, 12 occurred in ES naïve cases, since the clinical sites opted to perform GS without ES on these; our review determined that 11 of these 12 diagnoses *could have* occurred with clinical ES. Among the remaining 15 straightforward GS diagnoses that had prior nondiagnostic ES, we estimate that only four *could not* have occurred with ES, since these were due to variants not easily detected on exomes, such as structural and noncoding variants. For the other 11, recent improvements in capture kits, analytic pipelines, and interim disease–gene associations *could have* resulted in these diagnoses on a current ES. Thus, only 9 of the 84 straightforward diagnoses could not have been readily achievable in clinical practice with ES alone.

The clinical genetics practices at Duke, Stanford, and Vanderbilt request reanalysis of nondiagnostic exomes by the commercial laboratories that had performed the ES originally.

Table 4 Comparison of UDN-driven investigations at the clinical sites to standard clinical genetics practice.

Characteristics/ investigations	UDN	Clinical practice
Participant characteristics	Refractory to multiple prior clinical and laboratory evaluations, and often ES negative	More likely to not have ES, may or may not have failed prior clinical evaluations
Time spent on pre-, post-, and face-to-face activities	Face-to-face time represents a minority of time required for clinical and research activities (record review, literature review, phenotyping, bioinformatics, variant curation, RNASeq, collaborative science, integration of all data)	Limited by clinical demands and financial constraints to a few hours for all activities
Equity in access: •Geographic access •Financial considerations	Accessible to all in USA and internationally[a] All eligible irrespective of finances	Regional access more likely[a] Financial considerations likely factor
Complementation/ supplementation of prior clinical data	Personalized, temporally concentrated, comprehensive N-of-1 clinical consultations/laboratory tests/imaging/procedures • Fills in phenotypic gaps and generates additional clinical information • Leads to clinical diagnoses, diagnoses on targeted testing and contributes to genomic diagnoses	Variable, less likely to be temporally concentrated and comprehensive Time and financially constrained in filling in gaps and obtaining new information
Innovative analyses of genomic data	Straightforward diagnoses on UDN sequencing • ES/GS (35% diagnostic yield) Research reanalysis of pre-UDN raw data from nondiagnostic ES (diagnostic yield of 43%) • Multiple other approaches to resolving prior ES negatives Dual analysis of UDN-generated genomic data by UDN core lab and clinical sites • Clinical site analysis led to additional genomic diagnoses (8%) Manual curation of research variants generated by clinical site and core lab genomic analysis RNASeq: Internal collaborations led to generation and analyses of RNASeq (contributed to diagnoses in 15%) New disease gene identification • 8% of genomic diagnoses were novel disease–gene associations • Can be pursued with internal collaborations	Straightforward diagnoses on clinical ES (diagnostic yield 25–30% in literature); GS less widely available Standard reanalysis of negative ES with same pipeline (diagnosis yield of 6.5% at Duke, Stanford and Vanderbilt), 10–15% in literature • Limited further options to resolve ES negatives Dual analysis unavailable due to lack of bioinformatics in clinics Clinicians do not receive research variants from clinical labs for curation Limited availability of RNASeq, with the clinical laboratory determining access New disease gene identification • Time and resource constrained

ES exome sequencing, *GS* genome sequencing, *UDN* Undiagnosed Diseases Network.
[a]See Fig. S2 for detailed travel data.

This standard clinical reanalysis yielded diagnoses in 2/28 (7%) at Duke over four years, 6/83 (7%) at Stanford over four years, and 0/10 at Vanderbilt over one year (the Vanderbilt genetics clinics started requesting reanalysis only in the last year). In contrast, the UDN diagnosis rate with reanalysis of nondiagnostic ES over four years ($n = 23/53$, 43%) was significantly higher (Fisher's exact test, $p < 0.001$).

Data from Duke, Stanford, and Vanderbilt indicated that the travel distances to the UDN were qualitatively much greater than to their general genetics clinics (Fig. S2). This indicates that the UDN serves a geographically broader constituency than the general genetics clinics at the participating sites.

DISCUSSION

The mission of the UDN is to evaluate individuals with mystery illnesses and to provide innovative insights into rare and undiagnosed diseases. Applications are reviewed and acceptance is determined using the same inclusion criteria across clinical sites within the network. If indicated on medical record review, recommendations for additional clinically available testing are provided to the referring physician prior to acceptance, maximizing network resources for more challenging patients. UDN applicants accepted for participation are clinically and etiologically heterogeneous, with the underlying disorders including various rare and ultrarare genetic diseases as well as nongenetic disorders. The UDN clinical sites have developed a systematic, innovative, comprehensive, and reiterative diagnostic paradigm, outlined in the UDN manual of operations, and the evaluation is personalized to each participant. Critical components include the reconsideration of prior clinical and genomic data, filling in phenotyping gaps, generating and analyzing new clinical and genomic data, and working interactively with researchers inside and outside the UDN. The network interface allows clinical hypotheses to be rapidly moved to exploration and the infrastructure allows for facile data sharing for case matching and functional assays (in vitro molecular studies and animal

modeling). It is noteworthy that most of these UDN-driven investigations entail research activities that are difficult to achieve in standard clinical practice, with multiple avenues being necessary for diagnostic resolution in the majority of participants (Fig. 1). Therefore, the UDN is unique in complementing its N-of-1 patient-facing activities with cutting-edge research, providing a model for precision and translational medicine.

The N-of-1 model adopted by the UDN is not the traditional method utilized for disease gene discovery, but the network has been successful in identifying and pursuing candidate genes for ultrarare disorders. Internal and external collaborative science initiated at the four studied clinical sites resulted in 17 disease gene discoveries through case matching, animal modeling, and other molecular studies. Indeed, 7% of the 231 diagnosed individuals were found to have a novel disease. Several of the new disease–gene associations have resulted in establishment of patient foundations for advocacy and further research into pathophysiology and therapeutics.

The UDN diagnoses both genetic and nongenetic diseases (e.g., anti-HMGCR myopathy, Table 2 and Schnitzler syndrome, Table S1) through its participant-centric deep phenotyping. Of the 240 diagnoses, 45 (19%) were due to clinical synthesis of data or due to phenotype-directed testing. Although some of these diagnoses were tractable in a clinical setting, they had been missed previously by clinical diagnostic services, sometimes by multiple institutions: it is often difficult to know prior to evaluating a patient if a diagnosis could have occurred in standard clinical setting. The keys to these diagnoses were (1) a personalized planning of evaluations to fill phenotypic gaps and obtain additional information; (2) a temporally concentrated suite of specialty consultations, imaging, and laboratory tests, often within a week; and (3) synthesis of the emerging data by the specialists and the primary UDN investigators (medical geneticists, other clinicians, bioinformaticians, research genetic counselors, etc.). This N-of-1 precision medicine model of the UDN provides diagnostic power beyond what is available in clinical settings or in other research studies focused on cohorts. Furthermore, UDN clinical sites keep unsolved cases open indefinitely; diagnoses can occur years after individuals complete their evaluation[28] due to many reasons, such as reanalysis of data and adopting new technologies such as RNASeq. Thus, more than the present 231 of 791 individuals in this cohort may be diagnosed with time.

For every UDN participant suspected to have a genetic disease, the capabilities and limitations of prior genomic testing are considered. For example, early ES had lower coverage compared with current ES, and clinical laboratories are less likely to report novel candidate gene variants or variants in disease genes that are inconsistent with the phenotype.[28,37] The decision regarding further genomic testing is dependent on such considerations. For example, CMA was utilized if a prior CMA was not done or was on an outdated platform and provided five diagnoses that were due to large copy-number variants (CNVs) (95 Kb–3 Mb), which are most optimally detected by CMA, rather than next-generation sequencing such as GS. The UDN sequencing core provides clinical reports consistent with American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) reporting guidelines, and additional research variants that may warrant further investigation but do not meet the core's criteria for clinical reporting; these include coding and noncoding variants, structural variants, and trinucleotide repeats. Supplementing this report is the labor-intensive and evolving research bioinformatics output at the clinical sites, a unique UDN-driven investigation. Variants forthcoming from these dual analyses are pursued at the clinical sites, by extensive manual curation, functional assays, etc., contributing to increased diagnostic sensitivity. In addition, the clinical sites' research reanalysis of pre-UDN nondiagnostic ES data results in previously missed diagnoses and novel candidate gene identification (23 of 53 reanalyses, 43%) at rates that are much higher than reported in the literature.[22–24] Prior nondiagnostic ES are also solved with nongenomic approaches, rather than always moving on to GS for all such cases (Fig. S1). Successful resolution of these ES nondiagnostics allows conservation of UDN sequencing resources for other refractory cases.

The UDN diagnosis rate of ~30% might seem similar to that achieved with ES in standard genetics or exome clinics, but it is to be emphasized that these diagnoses occur in the context of high frequencies of prior nondiagnostic ES and non-diagnostic clinical evaluations, often at multiple tertiary centers. As hubs for undiagnosed disease data generation and integration in the UDN, the clinical sites can perform many investigations beyond those of a general genetics clinic. The personalized, compressed, iterative, comprehensive, and multidisciplinary UDN evaluations are difficult to achieve in standard clinics. We recognize that most of the straightforward diagnoses (35%, 84 of 240) made by the UDN ES and GS could be achievable in standard genetics clinics. Although GS is not yet standard of care in clinical practice, we elected to include the GS diagnoses in this calculation to provide a more conservative estimate, since many of the straightforward diagnoses made on GS could have occurred with ES, and it is likely that GS will become more broadly available to clinicians in the near future.

However, the remainder (65%, 156 of 240) of diagnoses required multiple UDN-driven investigations initiated at the clinical sites, such as the research reanalysis of raw data from prior nondiagnostic ES and the dual analysis of genomic data that are unique to the UDN and unavailable in genetics clinics, beyond reanalysis that may use the same standard clinical pipeline.[22,24] Furthermore, clinicians are unlikely to receive a list of research variants from laboratories that perform clinical ES for curation; the laboratories may include VUS in known disease genes or candidate genes, but only if there is some evidence for an association. Indeed, 39 of 190 (21%) genomic diagnoses in this study occurred directly due to the genomic sequence analysis at the clinical sites. Notably, the UDN has a mandate to accept individuals without regard

to their insurance or financial status; therefore geographic and financial barriers are minimized beyond what can be achieved in clinical practice, to improve equity in access to those with the most diagnostically intractable diseases. As a result, individuals who have no access to clinical ES or are denied ES coverage by insurance continue to be accepted by the network,[38] leading to some diagnoses that could be tractable in a clinical setting. This number is declining as clinical reimbursement for ES increases, although it is unlikely to ever become zero. However, we do anticipate increasingly more participants will enroll in the UDN with a prior nondiagnostic ES (current rate is ~75%, unpublished data). Hence, the proportion of straightforward diagnoses is likely to decrease, with more diagnoses requiring UDN-driven investigations. Unlike genetics clinics, the UDN also provides resolution to nongenetic diseases and can explore new methods such as long-read sequencing and optical mapping of the genome in refractory phenotypes, techniques that are still at the frontier of genomic research. Finally, unlike in most clinical settings, the UDN also standardizes its data, generating Human Phenotype Ontology terms[39] for the phenotype and sharing genomic data internally and externally, for further larger-scale cohort-type research studies.

Looking toward sustainability of this network, various models have been proposed, including adding more sites to decrease travel burden/costs; creating a tiered system to identify cases requiring additional research resources; scaling down the evaluations to only the most pertinent; greater use of telemedicine to decrease travel costs; and exploration of alternative funding sources. However, each of these have inherent limitations, and network discussions are ongoing.

Certain elements of the clinical sites' investigations could potentially be implemented into clinical practice. Clinicians could ask laboratories for a list of candidate genes and for variants in disease genes that were not reported due to poor phenotypic fit when ES reports are nondiagnostic. If there is discordance between a laboratory report and the clinician's interpretation, further discussions with the laboratory for possible reasons (such as alternative transcripts)[40] may result in resolution. Clinicians could periodically curate VUS through population and disease databases, apart from waiting for ES reanalysis by the original testing laboratory. Candidate gene follow-up is feasible in clinical settings, when successful case matching and collaborations occur without extended efforts. However, time and reimbursement constraints and lack of network expertise and resources are barriers to clinicians performing the extensive activities of the UDN clinical sites.

Our study has limitations. The retrospective design could have resulted in recall bias in the clinical sites' assessment of the UDN-driven activities that contribute to diagnoses, and it is possible that for some diagnoses the level of assertion may change over time, with additional data. Lack of objective data from the general genetics clinics at the UDN sites for many variables (such as data on other research collaborations that the genetics clinics may have established for nondiagnostic ES) precluded more direct comparisons. We also do not have the time interval between the original ES and the reanalysis for the nondiagnostic ES (both from the UDN cohort and the corresponding genetics clinics), thus tempering this comparison, since longer time intervals could result in higher diagnostic rates. We do not describe the role of internal collaborators such as the MOSC in detail (publications listed in Table S3), since the focus was on the clinical sites.

In conclusion, the UDN is a unique research network that directly benefits patients and simultaneously conducts rigorous research. The UDN clinical sites are integral elements of the network, incorporating critical research into the clinical evaluations to obtain incremental diagnoses and pursuing novel candidate genes to causality through collaborative science. Our analysis indicated that 65% of the UDN diagnoses would not be achieved in typical clinical settings. UDN-driven investigations inform the science and practice of genomic medicine. Even as both the clinical and research milieus are rapidly evolving with emerging technological advances, the network has the expertise and infrastructure to be on the frontier of new diagnostic paradigms for rare and ultrarare diseases.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-00984-z) contains supplementary material, which is available to authorized users.

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DISCLOSURE

P.L. is an employee of Baylor College of Medicine and derives support through a professional services agreement with Baylor Genetics, which performs clinical genetic testing services. M.T.W. is a stockholder of Personalis Inc. D.B.G. is a founder of and holds equity in Q State Biosciences and Praxis Therapeutics, holds equity in Apostle Inc., and serves as a consultant to AstraZeneca, Gilead Sciences, and GoldFinch Bio, outside the submitted work. The other authors declare no conflicts of interest.

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