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The National Institutes of Health undiagnosed diseases program

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Abstract

Purpose of review—To review the approach to undiagnosed patients and results of the National Institutes of Health (NIH) undiagnosed diseases program (UDP), and discuss its benefits to patients, academic medical centers, and the greater scientific community.

Recent findings—The NIH UDP provides comprehensive and collaborative evaluations for patients with objective findings of disease whose diagnoses have long eluded the medical community. Intensive review of patient records, careful phenotyping, and new genomic technologies have resulted in the diagnosis of new and extremely rare conditions, expanded the phenotypes of rare disorders, and determined that symptoms are caused by more than one disorder in a family.

Summary—Many children and adults with complex phenotypes remain undiagnosed despite years of searching. The most common undiagnosed disorders involve a neurologic phenotype. Comprehensive phenotyping and genomic analysis utilizing nuclear families can provide a diagnosis in some cases and provide good ‘lead’ candidate genes for others. A UDP can be important for patients, academic medical centers, the scientific community, and society.

Keywords

genome sequencing; phenotyping; undiagnosed diseases program

INTRODUCTION

The National Institutes of Health (NIH) undiagnosed diseases program (UDP) was initiated in May 2008 to address the unmet needs of individuals and families with rare or multisystem diseases who remained undiagnosed after an exhaustive evaluation; the diagnostic odyssey averages more than 5 years for most pediatric patients in the program. This review will focus on the successes, challenges, and lessons learned from the NIH UDP that may assist other academic centers in establishing similar programs. In addition, we illustrate our

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Conflicts of interest

There are no conflicts of interest.

fundamental belief that the study of rare and undiagnosed diseases leads to new disease discovery and enhanced understanding of disease mechanisms that can have implications for more common disorders.

LAUNCH OF THE NATIONAL INSTITUTES OF HEALTH UNDIAGNOSED DISEASES PROGRAM

Data from the NIH Office of Rare Diseases Research (ORDR) indicate that individuals with rare disorders (defined as affecting fewer than 200 000 persons in the United States) take 1–5 years to reach an accurate diagnosis in 33% of cases and greater than 5 years in 15% of such patients. Indeed, 6% of inquiries to the ORDR were from persons who were still searching for a diagnosis [1]. In May 2008, with \$280 000 in seed funding from ORDR, the National Human Genome Research Institute Intramural Program, and the NIH Clinical Center, the UDP began accepting applications with three major objectives: to provide patients who had been exhaustively evaluated with an accurate diagnosis, to use new genomic technologies to facilitate new disease discovery, and to elucidate new biologic pathways that would lead to a greater understanding of human physiology and more common disorders [2^{''}].

The response was overwhelming and underscored the magnitude of the unmet need. In the 6-year pilot phase, the program received over 10 000 inquiries, carefully reviewed over 3300 medical records, and 800 patients traveled to the NIH Clinical Center in Bethesda, Maryland, USA for week-long intensive evaluations that averaged 6–10 consultations and scores of tests. Approximately 40% were pediatric patients and approximately 30% of adult patients reported the onset of symptoms before age 18 years.

CRITERIA FOR THE EVALUATION OF PEDIATRIC APPLICANTS

Pediatric applicants peaked in two groups: infants and toddlers with congenital onset of symptoms and teens with symptom onset during early school age (Fig. 1). Nearly all had been evaluated by multiple subspecialists at local academic medical centers and many had traveled to major centers throughout the country. A complete medical record was compiled for each applicant and included: a detailed letter from the referring physician summarizing the evaluation and medical decision-making; copies of original diagnostic laboratory reports, radiographic procedures, biopsies, and special studies; operative reports and anesthesia records; and admission and discharge summaries. Electronic copies of radiographic studies and biopsy slides were also obtained for review by experts and, for pediatric patients, photos or videos of the affected child(ren) and family members, the newborn discharge summary including newborn metabolic screen results, and any relevant prenatal records were requested. Gathering the records required a major time commitment on the part of the parents, the referring physician, and UDP staff, and often took weeks to months to complete.

The cumulative medical record (often several hundred pages in length) was then sorted by discipline, electronically scanned, and reviewed by at least two, but often three or four, subspecialists relevant to the symptom constellation of the applicant. Subspecialists recruited from the faculty within the intramural NIH volunteered their time for the review of

applicant charts and evaluation of accepted patients. Each consultant note and diagnostic laboratory report was reviewed. We learned that the electronic medical record (EMR) can be a time-saver for documentation purposes, but can lead to two devastating errors: sloppy documentation and failure to take a fresh history or perform an updated comprehensive physical examination; and reliance on documentation of 'normal' in a medical note rather than reviewing the actual diagnostic laboratory report. Both of these types of errors permitted the UDP reviewers to recognize in several cases that a diagnosis had been made but overlooked by the referring team. A written summary was prepared by each reviewer detailing the extent of the workup, any relevant areas that had not been investigated, and a recommendation to accept or reject the applicant. Each case with recommendations was presented by the primary reviewer at a monthly screening meeting, attended by 15–20 NIH subspecialists, in which the final disposition on the application was made. Reviewers were also asked to recommend additional avenues of investigation whether or not applicants were accepted. In this way, many referring physicians whose patients were not accepted into the UDP received a personalized letter providing suggestions for further evaluation.

With expertise in rare and genetic disorders, the UDP staff evaluated pediatric patients with anomalies in multiple organ systems, congenital or progressive neurologic disorders, and neurodegenerative diseases. More than 50% of accepted pediatric patients had a neurologic phenotype, emphasizing that the physiology of the brain and nervous system remain poorly understood [2¹¹]. Special consideration was given to families with multiple similarly affected sibs.

Cases accepted by the program fell into several categories: the disease was extremely rare; the disorder was a rare presentation of a more common disease; the symptom constellation represented more than one disease; or the disease was indeed novel. In order to leverage the full power of next-generation sequencing, the program obtained blood from immediate-family members whenever possible, including both biologic parents and unaffected siblings.

PHENOTYPING THE PEDIATRIC PATIENT

Patients accepted into the program were notified and scheduled for week-long inpatient evaluations. Each admission required a 2–3-month 'lead time' and was the culmination of exhaustive review of recent records, current medications and nutritional requirements, and special travel considerations for medically fragile children. Clinical research nurse specialists prepared a comprehensive database for each child extracted from the medical record and conversations with parents and the medical home, and a daily schedule of evaluations was prepared based on the recommendations from the NIH reviewers. For patients with a neurological phenotype, the evaluation included many of the consultations or studies detailed in Fig. 2 and Table 1 [3¹²]. For the majority of the pediatric patients, who were either too young or too impaired to fully cooperate, a great deal of information was gathered during a 'sedation day'. With constant monitoring by a pediatric anesthesiologist, patients underwent clinically indicated studies often including brain MRI/magnetic resonance spectroscopy, lumbar puncture, skin biopsy, audio-evoked brain response, dental exam and cranial morphometry measures, and ophthalmologic exam under anesthesia. If not yet completed, additional blood was obtained for laboratory and research studies, and the

remainder of the physical exam, including dysmorphology measurements relevant to the phenotype (head circumference and shape; eye, ear, and philtral lengths; finger and palm length and palmar crease configuration; body proportion measures such as span and lower segment; and careful genital exam), was performed. Occasionally, impacted cerumen or nonfunctioning tympanostomy tubes were removed to assist the family in the care of their child. With careful choreography and impeccable communication on the part of the primary nurse practitioner, all the necessary studies could be performed in a single 4–5 h sedation. To date, we have performed sedations on more than 60 pediatric patients without adverse events. Research samples taken at the time of clinically indicated testing can be invaluable for functional studies to validate new gene and disease discovery (see list below). Biobanking of samples for validation and functional analysis in new disease discovery are as follows:

1. cerebrospinal fluid
2. serum
3. plasma
4. skin biopsy for fibroblasts and melanocytes
5. isolated DNA and RNA
6. urine
7. autopsy specimens (if available)

Every UDP family, and every patient, is a research project. Despite weeks of communication with families and combing through the medical record, there have been many ‘surprises’ when the patient arrived for evaluation. A fresh history and physical examination are imperative on day 1, when patients are consented for research. Sometimes, the entire schedule changes or additional studies are deemed necessary in order to comprehensively phenotype a patient. Regular and ongoing communication among caregivers, sub-specialists, and parents is required throughout the evaluation. There is no place for preconceived ideas or medical ‘silos’ in the evaluation of a patient with an undiagnosed disease.

Most UDP patients are not able to be diagnosed with a known disease. However, many have phenotypes, candidate DNA variations, and other clues sufficient to warrant further work on a research basis. Therefore, the final key undertaking of the UDP is an effort to establish collaborations around individual families. In general, we attempt to find a subject expert corresponding to the findings of interest in any given family. Arranging a collaboration often involves material transfer and intellectual property agreements that, once established, allow for ongoing communication of clinical and research updates among all stakeholders. As the basic research to establish new disease causation may take years to accomplish, the number of such collaborations accumulates rapidly. Several hundred are currently maintained by the UDP, a major challenge requiring novel bioinformatics and informatics infrastructures.

GENETIC ANALYSIS

That only 7000 genes have been associated with human disease among the 23 000 known genes suggests that many genetic disorders remain to be elucidated [3¹¹]. More common genetic diagnoses can be recognized by a unique clinical phenotype followed by molecular confirmation using gene-specific sequencing. In some cases, a more common phenotype, such as intellectual disability or cerebellar ataxia, can be approached by sequencing a panel of known genes associated with that phenotype. For clinical presentations with overlapping phenotypes, more than one panel of genes may be required. However, sequencing panels are expensive clinical tests that in some cases exceed the cost of exome sequencing.

A second and more agnostic approach to diagnosis takes advantage of single-nucleotide polymorphisms (SNPs), single base pair changes from the reference sequences that are present in more than 1% of the population under study. This type of DNA hybridization array can examine the entire genome using SNPs that are spaced approximately every 3000 base pairs. The technique reveals copy-number variants indicating small deletions or duplications, mosaicism within the sample, and regions of identity by descent. When used with exome sequencing, the SNP array can point to areas of interest such as small deletions in a gene that may harbor a deleterious mutation on the opposite allele, or areas of homozygosity that may be inherited by one or more affected members of a sibship. Additional uses of the SNP array include a quantitative measurement of mosaicism, to confirm parentage, to identify regions of the genome that segregate with disease in a family, and to identify other copy-number variations such as double deletions, or large deletions or duplications [3¹²].

Technical advances in massive parallel sequencing have facilitated cost-effective exome sequencing for clinical diagnostics. Each exome generates approximately 20 000 variants from the human reference sequence, any one or combination of which could be disease causing. Maximizing the utility of exome sequencing in rare and new disease discovery in UDP patients requires sequencing parents and unaffected siblings of the proband whenever possible. This allows filtering of variants using computer-generated algorithms for Mendelian consistency. Such filtration can reduce the list of candidate variants substantially, and does not rely on population matching, correct identification of orthologous sequences and other assumptions intrinsic to other filtration strategies. A final ranking of candidate variants can incorporate pathogenicity estimates from a number of free and commercially available programs [4]. Resulting candidate variations can be further interrogated based on the known or suspected function and their possible relationship to the clinical phenotype. Exome sequencing may also reveal additional information such as carrier state for other rare disorders, increased susceptibility to cancer or cardiovascular disorders, or predisposition to late-onset neurologic disorders. It is imperative, especially when sequencing parents and unaffected siblings, that all family members be counseled about the possible outcomes of genetic sequencing in order to make informed decisions about what information they do or do not wish to receive (see Biesecker paper, this issue).

RESULTS

Clinical [Clinical Laboratory Improvement Amendment (CLIA)-certified] exome sequencing is becoming increasingly common, although not universally available for undiagnosed pediatric patients. The diagnostic success rate for known genetic disorders is approximately 25% [5] and likely reflects where on the diagnostic odyssey the testing is initiated. Increasingly, applicants to the pediatric UDP have already undergone clinical exome sequencing with negative results. A research or ‘discovery’ exome, in combination with SNP array, performed on our families has yielded results that reflect the underlying assumptions and mission of the UDP.

The disease is extremely rare

Two sibs (one deceased) with early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) were found on SNP array to have a small homozygous deletion in exon 7 of *MEGF10*. The deletion was smaller than would have been reported on clinical SNP array and was not apparent on exome sequence [6]. The first case of adducted thumb–clubfoot syndrome (Ehlers–Danlos syndrome, musculocontractural type I) in North America was described in a consanguineous family because of homozygous mutations in *CHST14* (unpublished observation).

The disorder is a rare presentation or expanded phenotype of a more common disease

Two brothers (one deceased) with lower extremity spasticity, peripheral neuropathy, ptosis, oculomotor apraxia, dystonia, cerebellar epilepsy, and progressive myoclonic epilepsy were identified on exome sequencing to have a novel homozygous missense mutation in *AFG3L2* at the site of hetero-oligomeric complex formation with paraplegin, the protein mutated in hereditary spastic paraplegia type 7 (SPG7), producing a uniquely blended phenotype [7]. Three siblings with brain hypomyelination, microcephaly, cognitive decline, and skill regression but without photosensitivity or progeria were found to have biallelic *ERCC6* mutations, expanding the phenotype of Cockayne syndrome [8].

The symptom constellation represented more than one disease

Adams *et al.* [9] described a phenotypically discrepant sib pair, who both presented with fasting-associated ketotic hypoglycemia and lactic acidosis. Clinical suspicion followed by exome analysis revealed cytosolic phosphoenolpyruvate carboxy-kinase deficiency because of *PCK1* mutations in both sibs, along with Smith–Magenis Syndrome because of *RAI1* mutation in the older sib, and *N*-methyl-D aspartate-receptor glutamate insensitivity because of *GRIN2B* mutations in the younger sib.

The disease was indeed novel and represents a new understanding of human biology

St Hilaire *et al.* [10] identified nine persons with calcifications of the lower-extremity arteries and hand and foot joint capsule calcification because of biallelic mutations in *NT5E*. This gene encodes CD73, the enzyme responsible for converting AMP to adenosine, now considered to play a major role in inhibiting ectopic tissue calcification. This metabolic pathway may also have implications for another rare disorder, pseudoxanthoma elasticum [11]. Two siblings presenting with multiple neurologic complications and a paradoxical

immunologic phenotype characterized by severe hypogamma-globulinemia but limited infections were found to have genetic defects in *MOGS*, the gene encoding mannosyl-oligosaccharide glucosidase (MOGS). This congenital disorder of glycosylation (MOGS-CDG, formerly CDG-IIb) had been previously described only once, in a case of neonatal lethality. Further work with the UDP siblings demonstrated that a shortened immunoglobulin half-life was the mechanism underlying the hypogammaglobulinemia. This led to a hypothesis that impaired viral replication and cellular infection explains the observed decrease in susceptibility to infections and suggests a potential benefit of using inhibitors of MOGS as a means of controlling viral infections more globally [12⁹].

THE VALUE OF AN UNDIAGNOSED DISEASES PROGRAM

A UDP has value on several levels: for the patient and family, for the institution, and for the advancement of medical science. Likewise, not having a diagnosis can be a liability for families as well as the medical profession. When a child (or adult for that matter) has an obvious medical disability, the first question by a well-intentioned onlooker is often, 'What's wrong with your child?' The response of 'the doctors don't know' is often met with the implication (if not the actual statement) that the parent is just not consulting the correct physicians, instilling doubt on the part of the parents regarding their diligence in seeking the cause of their child's illness. In the absence of a diagnosis, the possibility of directed treatment cannot be addressed. In some cases, patients may be given a partial or incorrect diagnosis simply to obtain needed community services. Without a diagnosis, reproductive risk counseling and further family planning is uninformed. In some cases, there is limited access to rehabilitative services through the educational system in which resources are scarce and NO DIAGNOSIS equals NO SERVICES. Without a definitive diagnosis, medical providers may become frustrated, distant, and more likely to conclude that patients are somatizing or that symptoms are out of proportion to the underlying disease. Healthcare providers may assign multiple diagnoses in series or in parallel in an attempt to explain the symptoms. Indeed, many patients come to the UDP with 'too many' diagnoses, but none that fully explains the phenotype. However, even in the absence of a definitive diagnosis, the UDP can provide helpful information to families still on the diagnostic odyssey (see list below) [3¹⁰]. Information for families of rare and undiagnosed diseases are as follows:

1. Keep copies of all records, electronic and paper, and organize them routinely, especially reports from specialty diagnostic laboratories.
2. Carry an updated emergency letter with concise history, current medications, allergies, names and contact information of physicians and other care providers, and your child's preferences.
3. Establish a medical home even if you obtain many second opinions.
4. Find a physiatrist (rehabilitation medicine physician) to coordinate rehabilitative care (physical, occupational, and speech therapy).
5. Advocate aggressively for your child with the school system regarding what services your child should receive. Consider using a legal advocate if necessary.

6. Explore parent support groups for unknown disorders [syndromes without a name (SWAN) or rare disorders; National Organization for Rare Disorders (NORD)].
7. Periodically check with providers (especially geneticists) for new diagnoses reported in the medical literature that might apply to your child.
8. Carve out time for yourselves as caregivers by engaging extended family members or respite care services.
9. Work at supporting and being attentive to well children in the family.
10. For the very sick or dying child, consider an autopsy as a final attempt to establish a diagnosis especially when there is a possibility of future pregnancies.

Lastly, a UDP can help subspecialists communicate and think ‘outside the box’. Such a program can foster cross-specialty research collaborations and add prestige to an academic medical center as forward-thinking and cutting edge.

WHERE DO WE GO FROM HERE: THE UNDIAGNOSED DISEASES NETWORK

The popularity of the UDP has far exceeded the expectations and the resources of the NIH UDP. In the face of increased demand and to facilitate scientific discovery, the NIH Common Fund has committed funds to expand the program to create the Undiagnosed Diseases Network (UDN) (<http://www.genome.gov/27550959>). The UDN is comprised of six additional clinical sites across the country, a coordinating center, and two sequencing core laboratories; additional core laboratories will be added in the near future. As each UDP patient and family becomes its own research project, additional funds have also been devoted to basic research to validate excellent candidate genes not previously associated with human disease; functional studies in cell culture or model organisms will be employed.

CONCLUSION

A UDP can reap benefits on many levels, demonstrated by the 6-year NIH UDP pilot project. Applicants with objective findings who have long sought a unifying diagnosis for their symptoms undergo a comprehensive evaluation that engages a variety of relevant subspecialists who communicate regularly throughout the evaluation, performed clinically indicated diagnostic studies and tests, and collect samples for later functional analysis. Next-generation sequencing of the patient and immediate-family members provides the power to filter variants for Mendelian consistency to elucidate strong candidate genes for further study. Collaborations with basic scientists with expertise in relevant areas provide functional analysis that can clarify the pathophysiology of disease.

For the subset of patients long in search of a diagnosis, a UDP can invigorate an academic medical center, contribute to new disease discovery, advance medical science, and, perhaps most importantly, offer hope to patients and their families.

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KEY POINTS

- Many patients with rare disorders (defined as affecting fewer than 200 000 persons in the United States) take up to 5 years to receive an accurate diagnosis and as many as 6% remain undiagnosed, prompting the initiation of the NIH undiagnosed diseases program.
- Careful review of the entire medical record and accurate phenotyping of each UDP patient and potentially affected family member are critically important for the interpretation of genomic analysis.
- Analysis of SNP array and whole-exome sequencing of the proband and immediate-family members for Mendelian consistency can reveal the known disease-causing genes or identify strong candidate genes that can be authenticated by functional studies using patient samples and animal models.
- An undiagnosed diseases program can invigorate an academic medical center, contribute to new disease discovery, advance medical science, and offer hope to patients and their families.

Age distribution of Pediatric Applicants to the UDP

FIGURE 1.

The graph indicates the age of pediatric patients at the time of application to the UDP. The first peak represents the congenital onset disease (applicants' age 4–6 years) and the second peak represents the onset of disease symptoms at early school age (applicants' age 16–18 years). The time between the onset of symptoms and the time of application indicates the lengthy diagnostic odyssey experienced by most UDP patients. UDP, Undiagnosed Diseases Program.

<p>Phentype^a Consultations Genetics/genetic counseling Neurology Endocrinology Immunology Rheumatology Dermatology Cardiology Neuropsychology Nutrition Rehabilitative Medicine</p> <p>Diagnostic laboratory investigations Complete blood count with differential and peripheral smear (red cell morphology) Comprehensive metabolic panel Urinalysis Thyroid-stimulating hormone, thyroxine Lactate/pyruvate Ammonia Amino acids (plasma and urine) Organic acids (urine) Acylcarnitine profile Total and free carnitine Lysosomal enzyme analysis (leukocytes or fibroblasts) Coenzyme Q (white blood cells) Purines and pyrimidines (urine) Peroxisomal panel (plasma) Oxysterols Methylmalonic acid and homocysteine (plasma) Copper/ceruloplasmin Transferrin isoelectric focusing N- and O-linked glycans (plasma) Oligosaccharides and free glycans (urine) Glycosaminoglycans (urine)</p> <p>Procedures Swallow study (aspiration) Abdominal ultrasound (hepatosplenomegaly) Skeletal survey (dysostosis, fractures) Bone density scan (non-ambulatory or growth failure)</p>	<p>Bone age Electroencephalogram Muscle biopsy (histology and enzymology) Nerve biopsy</p> <p>Considerations for “sedation day” 3T MRI/MR spectroscopy of the brain (spine if indicated) Skin biopsy Ophthalmologic exam Brainstem auditory evoked response Lumbar puncture for neurotransmitters, inflammatory markers, folate, bipterin and neopterin Dental exam Large blood draws Bladder catheterization for urine Dysmorphology measurements and other portions of the physical that are exam difficult to perform in an uncooperative awake child Electromyogram and nerve conduction studies (while emerging from anesthesia)</p> <p>Additional studies if clinically indicated Electron microscopy (EM) of WBC buffy coat for inclusion bodies EM of axillary skin biopsy for evidence of storage Stool for ova and parasites, occult blood, fecal fat Autoimmune antibodies Quantitative immunoglobulins T-cell subsets Conjunctival or salivary gland biopsy</p>
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FIGURE 2.

Evaluation of a pediatric patient with a neurological phenotype. ^aModified from [3[•]], Table 83-2.

Table 1Examinations and procedures performed under sedation when necessary^a

Procedures	Examinations
3T MRI/MR spectroscopy of the brain (spine if indicated)	Ophthalmologic exam
Skin biopsy	Dental exam
Brainstem auditory evoked response	Dysmorphology measurements and other portions of the physical exam that are difficult to perform in an uncooperative awake child
Lumbar puncture for neurotransmitters, inflammatory markers, folate, bipterin and neopterin	
Bladder catheterization for urine specimen	
Large blood draws	
Electromyogram and nerve conduction studies (while emerging from anesthesia)	

^a Modified from [3[■]], Table 83-2.