article

Diagnostic yield in the clinical genetic evaluation of autism spectrum disorders

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Purpose: Clinical geneticists are often asked to evaluate patients with autism spectrum disorders (ASDs) in reference to questions about cause and recurrence risk. Recent advances in diagnostic testing technology have greatly increased the options available to them. It is not currently clear what the overall diagnostic yield of a battery of tests, either collectively or individually, might be. The purpose of this study was to evaluate the diagnostic yield of a stepwise approach we have implemented in our clinics. **Methods:** We used a three-tiered neurogenetic evaluation scheme designed to determine the cause of ASDs in patients referred for clinical genetic consultation. We reviewed the results of our diagnostic evaluations on all patients referred with a confirmed diagnosis of autism over a 3-year period. **Results:** By using this approach, we found an overall diagnostic yield for ASDs of more than 40%. This represents a significant increase in the diagnostic yield reported just a few years ago. **Conclusions:** Given the implications of these diagnoses on recurrence risk and associated medical conditions, a targeted neurogenetic evaluation of all persons with ASDs seems warranted. We discuss the issues in the future implementation of a fourth tier to the evaluation with the potential for an even higher diagnostic yield. ***Genet Med*** **2006:8(9):549–556.**

Autism spectrum disorders (ASDs) are a collective of neurobehavioral conditions that share in common primary abnormalities of socialization and communication. Diagnostic criteria for autism include impairment of reciprocal social interactions, impairment of verbal and nonverbal communications, restricted educational activities, abnormal interests, and stereotypic behavior. The genetic basis of autism is undeniable. Studies suggest that ASDs best fit into the model of "multifactorial inheritance." These disorders exhibit complex inheritance with marked etiologic heterogeneity. They occur four times as common in males. There is 70% concordance in monozygotic twins, 90% if a broader phenotype is used. The overall heritability of autism has been stated as approximately 90%.[1–5]

Clinical geneticists are often asked to evaluate individuals with autism for a possible etiologic diagnosis. Consistently reported diagnoses from such evaluations include chromosome abnormalities, genetic syndromes, metabolic disorders, and teratogenic causes.[6–14] Multiple reports over the past several years have evaluated the efficacy (diagnostic yield) of a search for a cause.[15–21] Diagnostic yields reported in these studies have ranged from approximately 5% to 25%. The variance in the reported yields in these studies can be attributed to significant differences in the studies themselves. This includes phenotypic definition, test selection, evaluation protocols, and timing of the studies. In fact, one of these studies recently reported an 8% diagnostic yield even for patients with pervasive developmental disorders—children with autistic-like features who do not fully meet diagnostic criteria for autism.[15] In review of this collective of reports, several consistent themes emerge. First, a significant number of diagnoses can be made by history and dysmorphology examination alone. Second, carefully considered medical and genetic testing significantly increases diagnostic yield. Third, diagnostic yield increases with the aggressiveness of the evaluation.

There have been several concerted attempts at outlining a standard approach to the genetic evaluation of autism.[13,22–24] Review of these documents suggests that there is general agreement that high-resolution (prometaphase) chromosome studies and Fragile X studies should be part of the evaluation of all individuals with ASD. There is, however, no general agreement as to the role of neuroimaging studies in the evaluation of persons with ASDs. Also, it is notable that these cited organizational policy statement documents were written before the advent of many of the newer testing modalities. Although we are aware that updated policy statements are currently being developed, decisions as to an extended evaluation have not been published.

Much has been written about the dramatic increase in the reported occurrence of autism. There is no clear consensus on how much of this increase is the result of changes in diagnostic and reporting practices versus a true biologic increase in numbers. Regardless of the correct answer to this question, the practical impact has been a striking increase in the referrals for genetic evaluation for patients with ASD. It is increasingly common for clinical geneticists to be asked to evaluate patients

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with ASD in reference to questions about cause and recurrence risk.

For the past 4 years, we have been using a "tiered" neurogenetic approach to the evaluation of autism. The evaluations and testing in the higher (earlier) tiers were selected on the basis of the predicted incidence of the disorder in patients with ASD, the invasiveness of the testing, the potential of intervention, and the overall practicality of obtaining tests. In our experience, this approach has been met with a high level of acceptance from third-party payers and the families. For this study, we reviewed our clinical data for a 3-year period from 2002 to 2004. We evaluated the effectiveness of our diagnostic strategy in patients with ASD and estimated its diagnostic yield.

MATERIALS AND METHODS

Study design

We performed a retrospective search for the patients referred to us for clinical genetic consultation with the primary diagnosis of ASD for the years 2002 to 2004 inclusive. Patients selected for further review all had an Axis I diagnosis of ASD made by a qualified specialist in autism. We identified 32 patients who met these criteria. Because these patients were evaluated by one of the two of us, all had undergone systematic diagnostic testing using the evaluation approach we had developed (see "Evaluation Scheme"). We then reviewed the results of the diagnostic evaluations of patients seen during this time period. These patients were reported as either having no identifiable diagnosis or characterized by an etiologic diagnosis and what part of the tiered evaluation their diagnosis corresponded with.

Evaluation scheme

During the course of seeing and evaluating patients with ASD, we developed a carefully considered diagnostic approach (Table 1). This approach was derived from our clinical experience and published data (reviewed above) on identifiable diagnoses in individuals with ASD.[16–21] We decided to evaluate these patients in a stepwise (tiered) manner. For the first two tiers, we chose to organize our studies to correlate with that previously reported by Shevell and colleagues,[21] on the basis of our agreement with their diagnostic philosophy.

Table 1
Tiered evaluation scheme used in this study

Tier 1
(initial evaluation to identify known syndromes or associated conditions)
Dysmorphology/clinical genetics (including Wood's lamp) evaluation
- If specific diagnosis suspected, proceed to targeted testing
"Standard" metabolic screening
Urine organic acids and mucopolysaccharides
Serum lactic acid, amino acids, ammonia, and acyl-carnitine profile
Sensory screening (audiogram)
Rubella titers, if clinical indicators present
Tier 2
Prometaphase chromosomes (karyotype)
Fragile X DNA studies
Brain magnetic resonance imaging
EEG
Tier 3
MECP-2 gene testing
22q11 FISH
15 interphase FISH
15 methylation/15q11-13 FISH (Prader-Willi/Angelman)
17p11 FISH (Smith-Magenis)
Serum and urine uric acid
- If increased production, HgPRT and PRPP synthetase superactivity testing
- If decreased production, purine/pyrimidine panel (uracil excretion, xanthine, hypoxanthine)
Subtelomeric FISH panel (if IQ <50)

EEG, electroencephalogram; MECP, methyl-CpG-binding protein; HgPRT, hypoxanthine-guanine phosphoribosyl transferase; PRPP, phosphoribosylpyrophosphate; FISH, fluorescence in situ hybridization; IQ, intelligence quotient.

Tier 1

The initial assessment was a standard clinical genetic consultation (history and physical) to identify known syndromes or associated conditions. This included a dysmorphologic/clinical genetics examination with a Wood's lamp evaluation. If a diagnosis was suspected on the basis of this initial assessment, we proceeded to targeted testing. In addition, "standard" metabolic screening for urine mucopolysaccharides and organic acids, as well as serum lactate, amino acids, ammonia, and acyl-carnitine profiles, was performed. All patients had sensory screening (to rule out a child with suspected autism who actually just had a hearing loss). Last, rubella titers were performed only if clinical indicators were present.

Tier 2

The second tier included prometaphase chromosomes, brain neuroimaging (magnetic resonance imaging), and DNA studies for Fragile X. In addition, an electroencephalogram (EEG) was performed. If the prometaphase chromosome study results were normal, and the patient had been noted to have clinically significant pigmentary changes on physical examination, a skin biopsy was obtained and a karyotype was performed on cultured fibroblasts.

Tier 3

In patients for whom no diagnosis was found in the first two tiers, we proceeded to a third tier of evaluations. In this tier we included chromosome 22q11 fluorescence in situ hybridization (FISH) studies, methyl-CpG-binding protein (MECP)-2

gene testing, chromosome 15 studies (15q11 FISH, interphase centromeric FISH, and methylation), 17p11.2 FISH, subtelomeric FISH panel (if intelligence quotient <50), and serum and urine uric acid testing. If an increased production of uric acid was noted, then hypoxanthine-guanine phosphoribosyl transferase and phosphoribosylpyrophosphate synthetase superactivity testing were performed. If decreased production was found, we performed a purine and pyrimidine metabolic panel (uracil excretion, xanthine, and hypoxanthine levels).

RESULTS

The results of our study are summarized in Table 2. We started with the cohort of 32 patients ascertained as having been referred for genetic evaluation with a diagnosis of autism. In the first two tiers of the evaluation we identified one patient each with a hearing loss (and not autism), neurofibromatosis, tuberous sclerosis, and Sotos syndrome. The patient with tuberous sclerosis is particularly notable in that no external (cutaneous) features were seen on examination. However, neuroimaging identified classic brain findings of tuberous sclerosis. Reexamination (again with a Wood's lamp) after the brain findings were discovered did not identify any overlooked abnormalities. We found two patients with Fragile X syndrome. Two patients had chromosome abnormalities (46 XY, dup22[q12.1 q11.23] and 46 XX, del9[p24.1]). Thus, the diagnostic yield of the first two tiers (8/32 patients) was 25%. Two additional patients were identified in the first two tiers as having cerebral dysgenesis (ventral induction defects) as part of their evaluation. We think these patients also have a positive finding on their diagnostic workup (thus giving us a 10/32 or a 30% yield). However, to avoid controversy over whether these represent a true causal relationship, we chose not to include them in our "positives" for the sake of this report.

Table 2
Results of diagnostic evaluations (N = 32)

Tier	Test/procedure	Abnormal results	Comments
Tier 1	Dysmorphology	2	Neurofibromatosis
			Sotos syndrome
	Metabolic screening	0	
	Sensory screening	1	
	Rubella titers	0	
Tier 2	Chromosome studies	2	46 XY, dup (22)(q12.1 q11.23)
			46 XX, del (9)(p24.1)
	Fragile X DNA	2	
	Brain MRI	1	Tuberous sclerosis (cerebral dysgenesis [2])
	EEG	0	
Tier 3	MECP-2	2	Both females, phenotype nonsyndromic
			ASD, not Rett syndrome
	22q11 FISH	0	
	Chromosome 15 studies	1	46XY,trp (15)(q11.2q12) phenotype
			ASD
	17p11 FISH	1	Subtle features of Smith-Magenis
	Serum/urine uric acid	1	Unclassifiable, persistent low serum uric acid
	Subtelomeric FISH panel	0	

MRI, magnetic resonance imaging; EEG, electroencephalogram; MECP, methyl-CpG-binding protein; ASD, autism spectrum disorder; FISH, fluorescence in situ hybridization.

In the third tier of the evaluation, another five patients (16%) had a positive diagnostic workup. These diagnoses were a duplication of chromosome 15 [46XY,trp(15)(q11.2q12)], two patients with MECP-2 mutations, one patient with a 17p11 deletion, and one patient with persistently low serum uric acid levels. Reinspection of the patient with the 17p11 deletion showed what was believed to be subtle findings suggestive of Smith-Magenis syndrome not seen on the initial examination. We were not able to identify the primary metabolic defect in the patient with the persistently low serum uric acid levels. However, the levels were significantly below normal on repeated blood draws, leaving little doubt that he has some, as yet unidentifiable, inborn error of purine/pyrimidine metabolism.

The summary of these data gives a calculated total diagnostic yield of 13/32 or 41%.

CONCLUSION

The last several years have produced a dramatic increase in the number of tests available to the clinical geneticist. As these tests have been applied to the etiologic workup of patients with ASDs, a host of new diagnostic associations have been reported. These include subtle (previously undetectable) chromosome abnormalities, atypical expression (expanded phenotypes) of known genetic syndromes, newly discovered inborn errors of metabolism, and specific gene mutations.

Chromosomal abnormalities

Advances in cytogenetic technology including prometaphase and late interphase studies have become the standard for clinical testing. With the use of higher resolution techniques, many chromosome aneuploidies have been reported in individuals with ASDs.[6,25–30] In particular, deletions or duplications of 15q and 22q are frequently seen in patients with ASDs.[29,31–38] (Of note, interphase FISH has traditionally been needed for confirmation of isodicentric chromosome 15 duplication.) The well-described relationship between pigmen-

tary changes and chromosomal mosaicism has direct application in the evaluation of ASDs.[39,40]

The application of FISH greatly enhances detection rates in neurogenetic evaluations. Current clinical applications of FISH technology include single-locus FISH, subtelomeric FISH panels, and chromosomal microarray (CMA) studies. Single-locus FISH studies have been reported to be positive in patients with ASDs for 22q11, 22q13, and 17p11.2.[37,38,41–44] The addition of a 40-probe FISH panel specific to the subtelomeric regions of the chromosomes has had a quantum effect in the evaluation of mental retardation. More recently, there have been assessments of its applicability in patients with ASDs. One study found 1 of 10 patients with idiopathic autism and moderate mental retardation had a chromosome 2q subtelomeric deletion.[45] Not surprisingly, the conclusion from this study was that it was too early to recommend universal screening for subtelomeric deletions in all patients with autism. Other studies have also indicated a link between terminal deletions of 2q37 and autism.[46–49] (However, the possible association of this subtelomeric deletion with ASD has been questioned. Deletions, and duplications, of this locus occur frequently as an apparent normal polymorphism in the general population.) Subsequent studies have not found subtelomeric deletions in larger cohorts of children with ASD.[50,51] At present, then, there are not enough supporting data to conclusively determine the utility of subtelomeric FISH panels in ASDs. Still, this test panel is absolutely indicated in patients with intelligence quotients less than 50.[52] Because mental retardation is a common comorbid condition with ASDs, one could surmise that subtelomeric FISH studies be performed in the subgroup of patients with ASD and severe to profound mental retardation. However, the most recent study suggested that this may actually be a low yield endeavor.[50] To our knowledge, CMA studies have not been reported in cohorts with ASD with the exception of studies of 15q.

Genetic syndromes

The association of autism/autistic behaviors with phakomatoses (tuberous sclerosis and neurofibromatosis) has been known for some time.[53,54] Similar to the discussions concerning Fragile X syndrome and autism, there is not complete agreement about the nature of the cause–effect relationship. Regardless, the incidence of autism is significantly increased in these two disorders compared with that of the general population.

Newer diagnostic associations have been reported with subtle/atypical expression of defined genetic syndromes and autism. The concept of an "expanded phenotype" of recognizable syndromes has been emerging over the past decade. In recent years, genotypic changes at loci associated with recognizable syndromes have been reported in individuals with ASD, but with few, if any, of the somatic features typically seen with these conditions.[55] This includes Rett syndrome (MECP-2 mutations),[56–58] Smith-Lemli-Opitz syndrome (7 dehydrocholesterol enzymatic block),[7,59] Angelman syndrome (15 methylation and UBE3A abnormalities),[60–62] Smith-Magenis syndrome (FISH detectable 17p deletions),[7,43] Sotos syndrome (NSD1 mutations),[63] Banayaan-Riley-Ruvalcaba syndrome (PTEN mutations),[64,65] and ARX gene-associated disorders.[66]

Metabolic disorders

Several of the "classic" metabolic disorders have been associated with autistic behavior. Individuals with untreated phenylketonuria are often said to be autistic. With current programs in newborn screening, it is hoped that this is a cause of ASDs that we will no longer see. Children with mucopolysaccharide storage disorders look strikingly similar to those with the reported "autistic regressive syndrome" in the early stages of clinical symptoms. Previous studies on diagnostic yields in ASDs have typically included metabolic screening for these and other metabolic disorders such as aminoacidopathies, organic acidurias, urea cycle defects, and fatty acid oxidation disorders.[21]

The most rapidly expanding diagnostic associations with ASDs have been the identification of a variety of metabolic disorders in which phenotype appears to be simply that of ASDs. This includes disorders of purine and pyrimidine metabolism, sulfation disorders, neurotransmitters, and creatine metabolism (Table 3).[67–79] In addition, mitochondrial abnormalities have been reported in association with an autism phenotype.[80–82]

Single gene disorders

The association of Fragile X syndrome and autism is well documented.[28,83–91] Some debate still exists as to the exact nature of autistic behaviors in Fragile X. Yet it is clear that the Fragile X is a frequent finding in persons with ASDs. Other rare folate-sensitive fragile sites have been reported in autistic patients. Some (2q13, 6p23, 12q13) have only been reported in autistic individuals.[92–94]

This study affirms previous documentation of the utility of a comprehensive clinical genetic evaluation of individuals with the diagnosis of autism. Recent studies indicate that such evaluations are even applicable across the milder aspects of the

Table 3
Metabolic disorders exhibiting an autism phenotype

Disorders of purine metabolism
Adenylosuccinate lyase deficiency
PRPP synthetase superactivity
Disorders of pyrimidine metabolism
Dihydropyrimidinase deficiency
Cytosolic 5' nucleotidase superactivity
Unknown sulfation defect
Urine S-sulfocysteine
Disorders of GABA metabolism
Succinic semialdehyde dehydrogenase deficiency
Disorders of creatine metabolism
Guanidinoacetate methyltransferase deficiency

PRPP, phosphoribosylpyrophosphate.

spectrum of pervasive developmental disorders.[15] This study demonstrates a significant increase in the diagnostic yield over earlier reports of the evaluation of persons with ASDs. Our overall diagnostic yield was more than 40% in this report.

This was a retrospective, non-epidemiologic review. Still, it is an accurate reflection of the impact of autism as it presents to a clinical genetics practice. The patients in this study were selected only in that they were referred for a genetic evaluation. It is difficult to imagine what bias this might introduce. Our tiered system of evaluation is practical and reasonable. In our experience, it is well accepted by third-party payers and the patients and their families. Careful explanation of the process before beginning the evaluation seemed to greatly decrease obstacles in completing the evaluation.

On review of our experience and the data from this study, we identified several important insights into this protocol. One of the most important factors we discovered was the importance of accurate diagnostics on the "front side" of the evaluation. Our patient with the hearing loss highlights the importance of documenting normal hearing before making the diagnosis of autism and/or proceeding with a diagnostic workup. In addition, there still remains a large diversity in the way the diagnosis of autism is made. To avoid stigmatization and unnecessary evaluations, we have initiated an autism screening, diagnostic, and triage clinic. Only those patients accurately assessed as having autism are then directed toward our tiered genetic evaluation protocol. We also found that having a clinical geneticist as part of this "first contact" team is invaluable. Many straightforward genetic diagnoses have been made at the time of the initial visit.

For the past year we have used a modified protocol from that used in this study (Table 4). Changes were made by reviewing the outcomes of this study and in incorporating newer technologies. Although it is a little too early to make firm conclusions, our initial look at the numbers for the past year suggests that this will further increase the diagnostic yield to more than 50%. Insights we have gleaned and modifications we have made include the following.

1. We found cytogenetic studies to be the most likely to give an answer. This is in agreement with almost all previous reports. Cytogenetic testing should remain a component of the higher tier tests.

2. The introduction of CMA studies has tremendously increased the diagnostic yield in mental retardation. We have placed CMA testing in our second tier of our ASD evaluation scheme. This makes sense at all levels. It is in keeping with the high yield of cytogenetic studies as mentioned under point number 1. In addition, there is a good argument for the cost-effectiveness of this decision. Clinical CMA panels would subsume several of the recommended tests in our original protocol including 22q11 FISH, 15q11-13 FISH, 17p11FISH, chromosome 15 centromeric studies, and the subtelomeric FISH panel. In addition, these studies include hundreds to thousands of other probes not otherwise tested. The cost of a CMA study is considerably less than the cost of multiple individual probes. At our institution, in fact, it becomes less expensive to perform CMA studies once three or more individual FISH studies are requested.

Table 4
Modified evaluation scheme suggested by the authors

Pre-evaluation
Formal diagnosis of AS/PDD by appropriate specialist
Sensory screening (audiogram)
EEG, if questions
Tier 1
(initial evaluation to identify known syndromes or associated conditions)
Dysmorphology/clinical genetics (including Wood's lamp) evaluation
- If specific diagnosis suspected, proceed to targeted testing
"Standard" metabolic screening, if clinical indicators present
Rubella titers, if clinical indicators present
Prometaphase chromosomes (karyotype)
Fragile X DNA studies
Tier 2
Brain MRI
CMA studies
Will include 22q11 FISH, 15 centromeric rearrangements, 15q11-13 FISH, 17p11 FISH, subtelomeric FISH panel
Tier 3
MECP-2 gene testing (only in females)
Chromosome 15 methylation studies
Serum and urine uric acid
- If increased production, HgPRT and PRPP synthetase superactivity testing
- If decreased production, purine/pyrimidine panel (uracil excretion, xanthine, hypoxanthine)

CMA, chromosomal microarray; EEG, electroencephalogram; AS, Asperger syndrome; PDD, pervasive developmental disorder; MRI, magnetic resonance imaging; FISH, fluorescence in situ hybridization; HgPRT, hypoxanthine-guanine phosphoribosyl transferase; PRPP, phosphoribosylpyrophosphate.

3. For this study, we chose to include "standard" metabolic testing in the first tier to make a balanced comparison with previous studies. In this study, no patients were identified with metabolic disorders. In reviewing the results of other studies, we could not identify data to support this as a high-yield modality. As such, we modified our approach to perform standard metabolic testing only in the presence of clinical indicators.

4. To date, we have found no reports of males with an MECP-2 mutation with the sole phenotypic expression being ASD. In this study, the two patients identified with MECP-2 mutations were female. Thus, we suggest performing MECP-2 gene testing only in females with ASDs until such time that there are data to support otherwise.

Although we are excited about the significant improvements in the diagnostic yield of patients with ASDs, there still remains more than half of the patients without a unifying diagnosis. We predict that the addition of other tests to our evaluation pro-

Table 5
Potential tests for the fourth tier

Screening for folate-sensitive fragile sites
Sequencing of the *FMR1* gene
Neuroligin gene testing (included in X-linked mental retardation panel)
7 dehydrocholesterol
UBE3A gene testing
CDLK5 gene testing
PTEN gene testing
NSD1 gene testing
Mitochondrial gene sequencing
Advanced metabolic workup
- Bratton and Marshall test to screen for succinyl-AICA-riboside; HPLC to confirm suspected cases
- Urinary creatinine excretion
- GC-MS of organic acid extracts in urine
- HPLC of nucleotides in erythrocytes
- Urine S-sulfocysteine
- Urine gamma hydroxy butyric acid

HPLC, high-pressure liquid chromatography; GC-MS = gas chromatograph mass spectrometry.

tocol would further increase diagnostic success. Table 5 lists several other clinically available tests for conditions that have been reported in association with an ASD phenotype. It is unclear to what degree the addition of any of these tests would add to diagnostic yield. Ideally, a large-scale, multicenter collaborative study of the utility of these and other potentially informative tests is needed to answer this important question.

We acknowledge several controversies in our recommended approach to the clinical genetic evaluation of patients with ASDs.

1. Our testing protocol is reasonably aggressive. Some groups have not recommended as much testing.[22] Given the implications of these diagnoses on recurrence risks and associated medical conditions, we believe that an aggressive neurogenetic evaluation of all persons with ASD is warranted. We consider our diagnostic yield more than justification for this approach.

2. Many families with children with autism request an EEG. The impetus for these requests is the hope that the underlying diagnosis may actually be Landau-Kleffner syndrome, and thus a "treatable" form of autism. It has been argued that the low incidence of Landau-Kleffner makes using an EEG a standard part of the evaluation of ASDs impractical. Still, the incidence of epileptiform disorders in persons with ASDs has been reported to be as high as 65%.[95] Thus, an EEG seems to be medically important for either instance. As such, we have placed this study in the "pre-evaluation" arm of our protocol.

3. Likewise, some would question the rationale of routine neuroimaging in patients with ASDs. Our patient with tuberous sclerosis and no cutaneous findings provides a clear answer of the utility of this testing modality. In addition, two of our patients had subtle markers of cerebral dysgenesis. Although the identification of such markers does not provide complete insight into cause, we still find knowledge of such anomalies helpful to clinicians and families.[96]

This study supports the work of others documenting the utility of a clinical genetic evaluation of patients with ASDs. With our protocol, we expect more than a 40% diagnostic yield. For these patients, detailed diagnosis-specific genetic counseling can be provided. For those in whom no diagnosis is found, empiric recurrence risk counseling can be provided. Currently the literature suggests that the recurrence risk for siblings is 4% if the affected child is a female and 7% if the affected child is a male. If there are two affected children, the recurrence risk is 25%. Thus, clinical geneticists can provide significant and meaningful input into the case management of every individual with ASD/pervasive developmental disorder-not otherwise specified. As such, a genetic consultation should be considered for all such patients.

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