

SPECIAL ARTICLE

Bottlenecks in Molecular Testing for Rare Genetic Diseases

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Despite the impressive progress in our understanding of the genetic causes of genetic diseases over the past decade, molecular diagnosis for rare genetic disorders is still in its infancy, being slow, expensive, unreliable, insufficient, and ill-organized in many countries. This leaves the gap between the hype of the current genomic research and the hope for a simple genetic diagnosis too large for patients and families affected with genetic disease. The bottlenecks in the molecular testing for rare genetic disorders are discussed below. *Hum Mutat* 0, 1–4, 2008. © 2008 Wiley-Liss, Inc.

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BOTTLENECKS IN MOLECULAR TESTING

Diagnostic tests for genetic diseases can roughly be divided into cytogenetics (e.g., chromosome analysis), molecular cytogenetics (e.g., fluorescence in situ hybridization [FISH] and microarray testing), and molecular genetics (e.g., gene mutation testing). Whereas cytogenetic and molecular cytogenetic tests are widely available in the Western world, molecular testing for most rare genetic disorders is currently not yet available in many countries.

Several decades of intensive research on the molecular causes of human genetic diseases, largely driven by the human genome project, have resulted in an impressive list of molecular tests for genetic diseases. As a consequence, laboratories that offer molecular tests to patients and their families have been established in many countries in the Western world. Unfortunately, very few molecular tests are available in continents such as Africa, South America, and Asia. Even in the Western world there are huge differences between different countries with respect to accessibility, price, and quality of molecular diagnostic testing [Harris, 1997; Harris and Reid, 1997; Stemerding et al., 1997; Godard et al., 2003; Hopwood et al., 2003; Ibarreta et al., 2004].

The bottlenecks in such testing include the vast number of genetic diseases, the low number of samples per disease, the nature of the disease mutation often being a private mutation, the advanced technology needed to detect mutations, the high cost of testing and lack of reimbursement by governments and insurance companies, and the lack of an international organized network of diagnostic laboratories combining their portfolio of tests.

One of the major bottlenecks in a cost-effective setup of molecular diagnostics is the relative rareness of most genetic diseases, resulting in a small number of requests per disease per laboratory. Of the more than 2,000 genetic diseases listed in OMIM with a specific gene defect (www.ncbi.nlm.nih.gov/Omim/mimstats.html), there are only a few with a prevalence of more than 1/10,000 in the Caucasian population: hemochromatosis type

1 (HFE gene), beta thalassemia (HBB gene), hypercholesterolemia (LDLR gene), cystic fibrosis (CFTR gene), breast cancer (BRCA1 and BRCA2 genes), colorectal cancer (MLH1, MSH2, MSH6, APC, and MYH genes), fragile X syndrome (FMR1 gene), alpha-1-antitrypsin deficiency (PI gene), spinal muscular atrophy (SMN1 gene), and prelingual deafness (GJB2 gene) are the most common genetic disorders with gene frequencies varying between 1 in 250 and 1 in 10,000 (Table 1). As traditional genetic testing still is mainly organized at the national level with few samples crossing borders, the amount of genetic tests performed annually per disease is not only determined by the disease/mutation frequency, but also by the size of the country and the number of laboratories testing in that particular country for that particular disorder. As the United States contains 300 million inhabitants, the amount of molecular tests performed there is quite large. However, Europe, with an estimated population of 490 million people, currently contains 47 different countries. So European countries have about 10 million inhabitants on average, with a mean annual birth rate approaching 100,000 newborns. Because the frequency of most genetic disorders is below 1 in 10,000, not more than 10 neonates with a specific genetic disorder are born each year per country. Assuming 10 genetic laboratories on average per country, each laboratory has less than one positive case for most genetic diseases. As less than 500 positive tests per year are expected in the whole of Europe for the majority of genetic disorders, a single test laboratory per disease in the whole of Europe would be more than sufficient, even assuming a low percentage of positives. Ironically, the amount of tests performed for a given disorder is currently not determined by the amount of positives expected, but by the

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TABLE 1. Common Genetic Diseases (Frequency >1/10,000) and Their Diagnostic Tests*

Disease	Frequency	Monogenic mutation	Genes	Mutation analysis	Conclusion
Hemochromatosis type 1	1/400	1/250	HFE	2 common mutations	Easy/cheap
Breast Carcinoma	1/20	1/500	BRCA1 BRCA2	Mutations in 23 exons Mutations in 28 exons	Complicated/expensive
Hypercholesterolemia	1/500	1/750	LDLR	Mutations in 16 exons	Complicated/expensive
Colorectal Carcinoma	1/25	1/1,000	MLH1 MSH2 MSH6 APC MUTYH	Mutations in 19 exons Mutations in 16 exons Mutations in 10 exons Mutations in 15 exons Mutations in 16 exons	Complicated/expensive
Cystic fibrosis	1/2,500	1/2,500	CFTR	Common mutations	Easy/cheap
Prelingual deafness	1/1,500	1/4,000	GJB2	1 common mutation	Easy/cheap
Fragile X syndrome	1/5,000	1/5,000	FMR1	Only 1 mutation	Easy/cheap
Alpha-1-antitrypsin deficiency	1/5,000	1/5,000	PI	2 common mutations	Easy/cheap
Spinal muscular atrophy	1/10,000	1/10,000	SMN1	Only 1 mutation	Easy/cheap
Beta thalassemia	Variable	Variable	HBB	Only 1 exon	Easy/cheap

*The disease frequencies are those observed in the Caucasian population. The diseases are ordered according to decreasing gene frequency. CA, cancer.

percentage of negatives one is willing to accept. As subgroups of the population get screened [Dallapiccola et al., 2006] for mutations in CFTR (infertile males, couples going for in vitro fertilization [IVF], couples at preconception), FMR1 (mentally retarded), FMR2 (mentally retarded), and SCA (ataxia), the relative number of positives for these tests is less than 10% in most labs because the incidence of mutations is very low in the patient groups screened. Apart from the increase in the number of disease genes that have become available for genetic testing over the last decade, the increase in the total number of molecular tests performed is mainly due to these screening policies [Rueda and Briones, 2002; Schmidtke et al., 2005; Dallapiccola et al., 2006]. This is to some extent due to unwarranted commercial interests of some laboratories, but also to awkward reimbursement systems in some countries (e.g., the Netherlands and Belgium) that have a standard reimbursement fee for every test performed [Harris, 1997], so that several “easy” tests have to compensate financially for every “difficult” test.

The molecular technologies used to detect mutations currently only form a minor bottleneck in the diagnostic process, and have not changed significantly over the last decade, although the quality, speed automation, and speed have been improved greatly: sequencing still is the gold standard, SSCP and denaturing gradient gel electrophoresis (DGGE) have been replaced by DHPLC (WAVE), which is technically easier and detects a higher percentage of mutations, and the technology of the first hour Southern blotting has been substituted by several technologies able to detect deletions and duplications (e.g., MLPA).

The nature of the mutation is a third and major bottleneck: overall, genetic disorders are characterized by a multitude of disease-causing mutations, necessitating the analysis of the complete open reading frame of the gene when searching for the disease-causing mutation. Only a handful of diseases are caused by a single or a limited number of mutations: these “easy” tests typically include spinal muscular atrophy (SMN1), Charcot-Marie-Tooth Type CMT1A (PMP22), hereditary neuropathy with liability to pressure palsies (PMP22) and sickle cell anemia (HBB), apart from all dynamic diseases caused by amplified nucleotide repeats, including fragile X syndrome (FMR1), FRAXE (FMR2), Friedreich ataxia (FRDA), Huntington type 1 (HD), Kennedy disease (AR), myotonic dystrophy type 1 (DMPK), Haw River disease (DRPLA), and spinocerebellar ataxias (SCA1, 2, 3, 6–8, 10, 12, 17) as the most prevalent ones. Some of these “easy” tests have very limited clinical value: 5% of all prenatal molecular testing in Italy is for FMR2 [Dallapiccola et al., 2006], although only a handful of

patients have been identified worldwide. A few other disorders also fall into the “easy” test category because they are caused in many patients by common mutations (hemochromatosis type 1, alpha-1-antitrypsin deficiency, prelingual deafness, and cystic fibrosis), or because the disease gene is easy to analyze due to its limited number of exons (beta thalassemia). Also, screening for SCA is very common, although mutations in SCA genes are uncommon. Apart from these handful of easy tests, for the majority of genetic disorders every patient has its own private mutation. As the average number of exons in the human genome is approximately nine, and varies between one and more than 100, the diagnostic process for some genes can be painstaking. Luckily, most of the common genetic diseases, including hemochromatosis type 1, alpha-1-antitrypsin deficiency, cystic fibrosis, prelingual deafness, fragile X syndrome, and spinal muscular atrophy (Table 1), are characterized by a limited number of mutations, which makes them amenable to fast, cheap, and easy diagnostic screening. The other common disorders, hypercholesterolemia and breast and colorectal carcinoma, are characterized by the absence of common mutations. Whereas little molecular testing for hypercholesterolemia is performed, as the biochemical phenotype is preferred above the molecular genotype, BRCA1 and BRCA2 testing (>50 exons) for breast and/or ovarian cancer and MLH1, MSH2, MSH6, APC, and MUTYH testing for colorectal carcinoma (>50 exons) have become the technical cornerstone of diagnostic testing in most genetic laboratories.

A fourth problem in genetic testing is the cost of the tests. In Western countries the average cost of molecular testing is about 500 Euro/600 USD (average price of the tests performed by GENDIA, a diagnostic network offering more than 2,000 different molecular tests). In many countries, genetic tests are not paid for by the state or insurance companies, certainly not if the samples are analyzed in foreign laboratories. In that case, this is a heavy financial burden on patients and families affected with a genetic disease, as several tests are often performed, mounting to a considerable cost [Godard et al., 2003].

A fifth bottleneck is the large number of genetic diseases for which molecular tests are available, now more than 1,000 (www.genetests.org). As the United States contains several hundred molecular laboratories, the portfolio of molecular tests that are offered within that country is quite large (500–1,000). In Europe, the amount of tests available in a single country varies from a few hundred at the very best in countries with a well-developed service system—e.g., 300–500 genes in the UK (www.ukgtn.org/gtn) and the Netherlands (www.dnadiagnostiek.nl)—to only a few

dozen in many European countries (16). In Australia a few hundred molecular tests are available, whereas in most countries of the remaining continents, Africa, South America, and Asia, only a limited number of molecular tests are available. Overall, the majority of genetic disorders cannot be diagnosed within the country of the patient.

An evident sixth bottleneck is the strategy of most laboratories to opt for a portfolio of: 1) “common” tests as summarized in Table 1; 2) “easy” tests as tabulated in Table 2; and 3) “favorite” tests, specific tests in which the laboratory has some specific interest (commercial interest in commercial laboratories and research interest in research laboratories). Evidently, this has created a situation in which the portfolios of the different laboratories are very similar, leading to duplication of effort in respect to the few “common” or “easy” tests on one hand, and deficiencies in respect to the many uncommon diseases. So many laboratories located in the same country or even in the same region are competing for samples to test for the common diseases, each getting relatively few, whereas on the other hand it can be quite difficult to find a laboratory able to test for many of the rare diseases. Even when such a laboratory is found, especially when it is outside of the country, the turnaround time and financial requirements usually are uncertain. Furthermore, many laboratories providing genetic tests in Europe, Asia, and Australia still float between a diagnostic and research setting, with intermediate quality, price, and turnaround time, and lack of International Standards Organization (ISO) or Clinical Laboratory Improvements Act (CLIA) accreditation. There also exists little collaboration between laboratories within individual countries, and there exist no national network of laboratories allowing samples to be sent to a central location that functions as a clearinghouse, sending the samples to different test laboratories.

All these bottlenecks impair a cost-effective and reliable diagnostic service, thereby holding molecular testing in many countries in a preclinical era. However, the quality, accessibility, and cost-effectiveness of diagnostic tests for rare genetic disorders could be substantially improved by the creation of an international network of diagnostic laboratories, combining their portfolio of tests and exchanging samples as recommended by the European Society of Human Genetics [2003].

ADVANTAGES OF AN INTERNATIONAL DIAGNOSTIC NETWORK

Several organizations, including GENETESTS (www.genetests.org), EDDNAL (www.eddnal.com), ORPHANET (www.orphanet), and EUROGENEST (www.eurogentest.org), list laboratories that offer specific testing leading to a limited exchange of (mainly research) samples across borders. However, there are currently hardly any international networks of diagnostic laboratories.

Several of the bottlenecks described above could be avoided by such an international network of diagnostic laboratories [European Society of Human Genetics, 2003], certainly if these were to be organized with a central laboratory receiving all samples and invoicing all results (Fig. 1):

1. The accessibility to genetic tests would increase worldwide: in countries with already developed molecular diagnostics, a network facilitates testing for rare diseases not covered by local laboratories. In countries with a less developed system for genetic diagnosis, a network can provide access to many tests. Basically, every possible genetic diagnostic test can be made available worldwide if one of the network laboratories offers it.
2. The quality of genetic testing is improved by the concentration of many tests in fewer laboratories. Test quality is determined by the accuracy of the test (amount of mistakes made), completeness of the test (sequencing of all exons vs. analysis of a few common mutations), quality of the test report (clear explanation of methods, results, and implications), turnaround time (how long it takes to complete the test), and cost (defined as the cost of the test for the laboratory). As a network ideally only uses one or a few laboratories per specific test (Fig. 1), it is evident that every test laboratory participating receives more samples for each test, leading to improvement in test quality and test reporting, and reduction of turnaround time and cost.
3. The routing of samples is simplified and facilitated, and it is no longer necessary for referring clinicians or local laboratories to look for a laboratory to perform a particular test that cannot be performed locally, as all samples can be sent to a central laboratory in the network, which distributes the sample to the correct test laboratory, and issues a standardized English result report with postanalytical advice to the referring laboratory (Fig. 1).

TABLE 2. Genetic Diseases With Easy-to-Perform Diagnostic Tests*

Disease	Gene	Mutation
Fragile X	FMR1	Repeat
FRAXE	FMR2	Repeat
Friedreich ataxia	FRDA	Repeat
Haw River	DRPLA	Repeat
Huntington type 1	HD	Repeat
Kennedy	AR	Repeat
Myotonic dystrophy type 1	DMPK	Repeat
Spinocerebellar ataxia	SCA1,2, 3, 6, 7, 8, 10, 12, 17	Repeat
Alpha-1-antitrypsin deficiency	PI	2 mutations
Charcot-Marie-Tooth Type CMT1A	PMP22	1 mutation
Cystic fibrosis	CFTR	Common mutations
Deafness	GJB2	1 mutation
Hemochromatosis type1	HFE	2 mutations
Hereditary neuropathy with liability to pressure palsies	PMP22	1 mutation
Sickle cell anemia	HBB	1 mutation
Spinal muscular atrophy	SMN1	1 mutation
Beta thalassemia	HBB	1 exon

*The table contains dynamic repeat diseases characterized by a single mutation, the most frequent diseases with common mutations, and frequent diseases with only one exon.

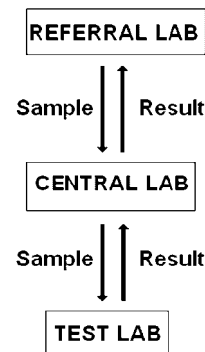


FIGURE 1. Flowchart of an international network. Samples go from the referring party through the central laboratory to the specific test laboratory for the required test, and result reports go from the test laboratory to the central laboratory, where they are transformed into standardized English results that are issued to the referring party.

AN INTERNATIONAL DIAGNOSTIC NETWORK SUPPORTING LOCAL GENETIC COUNSELING

Although the availability of an international network of diagnostic laboratories is a prerequisite for high-quality cost-effective genetic testing, such a network should only be seen in the context of improvement of local genetic counseling. The function of an international network of laboratories is not to provide unlimited over-the-counter testing of patients thereby bypassing the local circuit of genetic counselors, as the only purpose of genetic testing is to support genetic counseling of the patient and family. However, such a diagnostic network could facilitate genetic counseling in several ways, as outlined by the European Society of Human Genetics [2003]:

1. Facilitation of testing: The current local level of molecular testing with all drawbacks discussed above, insufficiently supports local genetic counseling as it does not provide cost-effective genetic testing, which is a cornerstone of the counseling process.
2. Preanalytical test advice: Only an international network of diagnostic laboratories will be able to provide an extended portfolio of a few thousand genetic tests, and allows choosing for the most appropriate test. The central laboratory of the network can help the referring laboratory or local genetic counselor in choosing the best test in a case-by-case approach based upon the specific symptoms of the patient tested, the cost of the different tests, and the detection rate of each test. Such a network can have many stand-by clinical geneticists and dysmorphologists available to help local counselors and clinicians with the clinical diagnosis and suggest the most evident flowchart of genetic tests. Such preanalytical advice is evidently essential in cost-effective testing.
3. Postanalytical test advice: The test report should contain appropriate recommendations in the case of positive (mutation identified) or negative (mutation identified) result. If a disease-causing mutation or a variant of unknown significance is identified, the implications should be discussed in the report. This can be done more efficiently by experts of an international network dealing with many of these tests than by a local laboratory dealing with a few of these tests yearly. If no mutation is identified, additional reflex testing is much more efficient when a complete portfolio of possible tests for any given disorder is available.

CONCLUSION

An international network of genetic diagnostic laboratories such as proposed here will result in greater access to a large spectrum of genetic tests performed with higher quality, lower turnaround time, and lower cost. Furthermore, such a network will not only provide top quality molecular testing, but also facilitate local genetic counseling of the patient. Evidently, such an international network can only function properly if the current protectionist attitude of genetic test laboratories, government, and insurance companies is replaced by an open attitude allowing samples to be tested abroad and patients and/or laboratories reimbursed [European Society of Human Genetics, 2003; Godard et al., 2003].

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