External quality assessment schemes in molecular genetic testing. EQA-PKU

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Quality in diagnostic molecular genetic testing, as well as in clinical laboratory testing in general, is of particular importance in modern health care. A number of programmes and mechanisms for its assurance, control and improvement are already in action or under development. Participation in external quality assurance (EQA) schemes as a prerequisite for accreditation of a clinical laboratory has become compulsory in a number of countries. At present, the most important source of EQA schemes for molecular genetic testing is European Molecular Genetics Quality Network (EMQN). To expand the list of EQA schemes available for European clinical laboratories performing molecular genetic testing and to improve quality assurance in molecular genetic testing in phenylketonuria (PKU), the joint research project "Molecular Genetic Testing in Phenylketonuria: a Model to Assess the Quality Control System for Monogenic Disease" (acronym MOLGENT) supported by EC INCO-COPERNICUS programme was prepared and implemented in 1999–2002. It resulted in optimal strategy and a set of standardised protocols for *PAH* gene mutation identification and a pilot scheme for EQA in molecular genetic testing in PKU, which is expected to be the basis in the establishment of a relevant EQA scheme within EMQN.

Key words: clinical molecular genetic testing, external quality assessment, *PAH* gene mutations, phenylketonuria, PKU

INTRODUCTION

One of the most rapidly developing fields in diagnostic laboratory testing is genetic testing, which is aimed to identify changes in gene(s) related to an inherited disease or congenital anomaly. In molecular genetic testing, definite gene changes are identified or ruled out directly or indirectly on nucleic acids level. An important aspect of molecular genetic testing is that such tests are almost exclusively qualitative (identification of the presence or absence of a mutation), while in other diagnostic laboratory tests quantitative ones prevail. Tremendous advances in human genome sequencing and further investigation of its structure and function [1] give a new impulse to the development and clinical application of molecular genetic testing.

Molecular genetic testing, being a subspecialty of clinical laboratory testing, must meet the general requirements of quality assurance applied to the total laboratory testing process in general and clinical laboratories in particular. Accreditation of clinical laboratories requiring to participate in the **schemes of external quality assessment** (EQA) are compulsory in many countries including EU. EQA, or proficiency testing, is a procedure for assessing and maintaining the quality and standard of output from a laboratory. It measures the error rate of the laboratory, helps to identify the underlying problems and leads to an increasing competitiveness between laboratories. On the other hand, results of the international EQA schemes influence national standards of clinical testing and urge diagnostic industry to improve production and to develop new methods in laboratory testing. The end result is improved performance and better quality control ensuring that patients and clinicians receive the best possible service. EQA schemes can be organised in different ways, but most often they are organised by specialised institutions, *e.g.*, Labquality [2] in Finland, UK National External Quality Assessment Scheme (UKNEQAS) [3] where individual laboratories can be providers of a definite scheme. The demand for

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clinical molecular genetics testing has been steadily growing since its introduction in the 1980s, leading to the necessity of relevant EQA schemes. National schemes were started in a number of countries (*e.g.*, assessing genotyping DNA samples for DMD, CFTR and HD run in UK, BRCA in Germany, etc.). EQA scheme for the identification of CFTR gene mutations causing cystic fibrosis, organised by the Cystic Fibrosis Genetic Analysis Consortium in 1996, became the first European international large-scale EQA scheme [4]. Variation in the performance of laboratories across Europe [5, 6] and the demand from European centres to be included in the schemes already in action led to the creation of the European Molecular Genetic Quality Network (EMQN) [7, 8]. EMQN is in the process of development by including new schemes (Table 1) and new members (from 14 in 1997 to 500 in 2001 [9]).

A wide variety of approaches and techniques for the identification of mutations and nucleotide sequence polymorphisms in different genes is available [11]. EQA schemes based on the comparability of performance in different laboratories would be impossible without standardisation and optimisation of testing necessary for maintaining and raising standards among laboratories, facilitating communication between separate laboratories and providing a guide for EQA assessors and participants. Therefore, best practice guidelines are being developed as an integral part of EQA (see EMQN website [7]).

Scientific research has revealed the molecular basis for several hundreds of inherited diseases, but only a small portion of them (predominantly monogenic ones) are tested for the presence of pathology causing mutations on clinical molecular genetics laboratory level. EQA schemes are available for an even more restricted group for monogenic diseases that are most commonly offered in diagnostic services (Table 1). At present, EQA schemes are being developed in two main directions: (1) new EQA schemes for relatively frequent diseases (**application-based proficiency testing**) and (2) **methodological proficiency testing** trials intended to control the quality of the elementary analytical steps in molecular genetic diagnosis.

To expand the list of EQA schemes available for European clinical laboratories performing molecular genetic testing and to improve quality assurance in molecular genetic testing in phenylketonuria (PKU) by developing a standardised and optimised testing scheme and protocols, the joint research project "Molecular Genetic Testing in Phenylketonuria: a Model to Assess the Quality Control System for Monogenic Disease" (acronym MOLGENT) supported by the EC INCO-COPERNICUS programme was prepared and implemented in 1999–2002 [12].

Table 1. **EQA schemes presented by the EMQN**

Scheme	1997 [5]	2001 [9]	2002 [10]
Huntington disease	+	+	+
Familial breast and ovarian cancer		+	+
Fragile-X syndrome		+	+
Prader-Willi / Angelman syndromes		+	+
Charcot-Marie-Tooth disease		+	+
Retinoblastoma		+	+
Duchenne muscular dystrophy		+	+
Friedreich ataxia		+	+
Cystic fibrosis		*	*
Y-chromosome microdeletions		**	**
Haemochromatosis***			+
DNA sequencing***			+

* Organised by the European Thematic Network for Cystic Fibrosis (http://www.cfnetwork.be/cfnetwork.htm).
** Organised by the European Academy for Andrology.
*** New proposed schemes.

SUBJECTS, MATERIALS AND METHODS

Four centres in Lithuania, Latvia, Germany and Italy participated in the collaborative study (see MOLGENT website [12]).

Molecular characterisation of the PAH locus in individuals with PKU. Probands and their families were selected for molecular genetic testing of the *PAH* locus according to the PKU diagnosis based on clinical, biochemical and genealogical investigation of the patients residing in Lithuania, Latvia, Germany and five regions of Italy. Clinical, biochemical and molecular genetic testing of the patients with PKU was performed according to the standardised scheme and protocols developed in the current research (available online [12]).

Standardisation and optimisation of molecular genetic testing in PKU. Optimised schemes for clinical and molecular genetic investigation in hyperphenylalaninemia and standardised protocols were developed in close collaboration of all partners. Standardisation was achieved on the basis of the experience of the partners (particularly from the Italian and German centres) in the field, scientific and methodical peer-reviewed publications and parallel testing of the DNA samples from Lithuania in Lithuanian and Italian centres.

Development of a scheme for external quality assurance in molecular genetic testing in PKU (EQA-PKU). The principles of the EQA-PKU scheme and workplan for its creation and implementation were developed and pilot trials of the developed EQA scheme were organised and performed following the EMQN examples.

RESULTS AND DISCUSSION

What makes PKU (MIM 261600) relevant for clinical molecular genetic testing? This deficiency of phenylalanine hydroxylase (PheOH; EC 1.14.16.1) is one of the most common severe inborn errors of amino acid metabolism in the white population over the world, with an average incidence of 1 : 10,000 [13]. *PAH* gene has been cloned and its nucleotide sequence is included into The Genome Database (GDB) [14]. Various mutations in the *PAH* gene have different effects on PheOH enzymatic activity leading to a range of clinical forms from mild hyperphenylalaninemia to severe PKU [15]. Phenylalanine hydroxylase locus knowledgebase (PAHdb) [16] and The Human Gene Mutation Database (HGMD) [17] include information on >400 different *PAH* nucleotide sequence polymorphisms (either related to hyperphenylalaninemia or neutral). The established genotype-phenotype correlation in a number of the *PAH* gene mutations [15] makes molecular genetic testing in PKU especially valuable in the precise diagnosis of PKU suspected on the basis of mass newborn screening data. Identified *PAH* genotype enables efficient prediction of the clinical form of PKU and individual selection of optimal treatment. Prenatal diagnosis in PKU is important to prepare the family at risk to face the problem in the case of inherited PKU.

The first stage in the development of an optimal scheme and a set of the most important standardised protocols was **molecular characterisation of the *PAH* locus** in PKU to reveal the spectrum and prevalence rates of PKU mutations. Investigation of the *PAH* gene mutations in the Lithuanian, Latvian, Italian and German populations in the present study was performed in line with the previous research (see [12] for the list of references); the results obtained in the present study are summarised elsewhere [18–20]. The vast majority of PKU causing *PAH* gene mutations are missense mutations with a smaller fraction of small insertions/deletions, frameshift and splicing mutations, and minor occurrence (1–3% or more, depending on population) of large deletions or other type mutations, which are undetectable using PCR amplification-based approach. Thus, the strategy for molecular genetic testing in ungenotyped cases of PKU and for carrier status should be based on the sequence-specific testing.

Choosing **optimal strategy for molecular genetic testing in PKU** depends on whether the test is aimed to genotype the ungenotyped patient with PKU (mutation scanning) or to test embryo / unaffected family member for the presence or absence of a known familial mutation (mutation identification). An optimal (but not the only possible one) scheme for mutation scanning in PKU was suggested based on PCR amplification and broad-range denaturing gradient gel electrophoresis (DGGE), either standard [21] or multiplex [22], of all exons of the *PAH* gene followed by identification of mutations through DNA sequencing of relevant exons and verification of the identified mutation using alternative sequence-specific approach. Exceptional prevalence of the R408W mutation in Latvia and Lithuania [19, 18] makes it reasonable to start molecular genetic testing in PKU from the direct identification of this mutation, using the standardised protocol (see below) in these populations. The suggested strategy for the identification of a definite mutation is based on the PCR amplification of a relevant exon of the *PAH* gene with a subsequent application of a definite sequence-specific method, preferentially the standardised one (see below). A set of standardised protocols for routine identification of 21 known relatively prevalent *PAH* gene mutations and key procedures in molecular genetic testing for PKU (multiplex DGGE of all *PAH* gene exons, automated DNA sequencing of *PAH* gene fragments) were developed (available online [12]).

Scheme for external quality assessment in molecular genetic testing in PKU (EQA-PKU) was developed following EMQN examples. Two pilot trials were organised under the MOLGENT project. Despite extensive information, only one external laboratory (Department of Human Genetics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine) applied for the participation in both trials. Results of the EQA-PKU trials showed the developed EQA scheme for PKU to be efficient and applicable, although too few data did not allow statistical analysis. The developed scheme is expected to be the basis in further establishment of a relevant EQA scheme within EMQN.

Concluding remarks. Clinical and genetic heterogeneity of monogenic diseases and a variety of methods applied in molecular genetic testing do not allow developing a “gold standard” and general scheme for routine molecular genetic testing in such diseases. Unless new generation technologies such as DNA chips are available for all clinical laboratories, individual testing schemes as well as EQA schemes for clinical molecular genetic testing should be developed on the basis of optimised strategy and protocols relevant for a definite disease, as well as the

main principles for quality assessment and assurance in clinical laboratory testing in general and molecular genetic testing in particular.

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References

1. International Human Genome Sequencing Consortium. Nature 2001; 409(6822): 860–921.
2. Labquality. 2002; http://www.labquality.fi
3. United Kingdom National External Quality Assessment Schemes (UKNEQAS). 2002; http://www.ukneqas.org.uk
4. Dequeker E, Cassiman J-J. Eur J Hum Genet 1998; 6 (2): 165–75.
5. Losekoot M, Bakker B, Laccone F, Stenhouse S, Elles R. Eur J Hum Genet 1999; 7 (2): 217–22.
6. Dequeker E, Cassiman JJ. Nat Genet 2000; 25 (3): 259–60.
7. The European Molecular Genetics Quality Network (EMQN). 2002; http://www.emqn.org
8. Müller CR. Eur J Pediatr 2001; 160 (8): 464–7.
9. EMQN Newsletter 2002; 8 (January/February).
10. EMQN Newsletter 2002; 9 (April/May).
11. Elles R (ed.). Molecular Diagnosis of Genetic Diseases. Totowa, 1996.
12. MOLGENT website. 2002; http://www.geneticahumana.lt/molgent.htm/
13. Online Mendelian Inheritance in Man, OMIM™. Phenylketonuria. MIM Number: 261600.
14. The Genome Database (GDB). 2001; http://gdbwww.gdb.org/gdb/
15. Guldberg P, Rey F, Zschocke J et al. Am J Hum Genet 1998; 63 (1): 71–9.
16. Phenylalanine hydroxylase locus knowledgebase (PAHdb). 2002; http://ww2.mcgill.ca/pahdb/
17. Human Gene Mutation Database (HGMD). Institute of Medical Genetics in Cardiff. 2002; http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html/
18. Kasnauskienė J, Giannattasio S, Lattanzio P, Cimbalistienė L, Kučinskas V. Hum Mut (in press).
19. Pronina N, Giannattasio S, Lattanzio P, Lugovska R. Hum Mut (in press).
20. Giannattasio S, Dianzani I, Lattanzio P et al. Hum Hered 2001; 52 (3): 154–9.
21. Guldberg P, Henriksen KF, Güttler F. Genomics 1993; 17 (1): 141–6.
22. Michiels L, Francois B, Raus J, Vandevyver C. J Inherit Metab Dis 1996; 19 (6): 735–8.

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IŠORINĖS MOLEKULINIŲ GENETINIŲ TYRIMŲ KOKYBĖS ĮVERTINIMO SCHEMOS. EQA-PKU

S a n t r a u k a

Molekulinių genetinių tyrimų kokybė, kaip ir diagnostinių laboratorinių tyrimų kokybė apskritai, ypač svarbi sveikatos apsaugoje. Jau veikia ar dar kuriama daugelis jos užtikrinimo, kontrolės ir gerinimo programų bei mechanizmų. Dalyvavimas išorinėse kokybės įvertinimo (IKĮ) schemose, kaip būtina klinikinės laboratorijos akreditavimo sąlyga, tapo privalomas daugelyje šalių. Šiuo metu svarbiausias molekulinių genetinių tyrimų IKĮ schemų šaltinis yra Europos molekulinių genetinių tyrimų kokybės tinklas (EMQN). Europos klinikinėms laboratorijoms, atliekančioms molekulinius genetinius tyrimus, prieinamų IKĮ schemų sąrašui išplėsti ir fenilketonurijos (FKU) molekulinių genetinių tyrimų kokybei pagerinti buvo parengtas ir įgyvendintas jungtinių tyrimų projektas „Molekuliniai genetiniai fenilketonurijos tyrimai: monogeninių ligų kokybės kontrolės sistemos kūrimo modelis“ (1999–2002). Jį vykdant parengta optimali strategija ir standartizuotų protokolų rinkinys *PAH* geno mutacijoms nustatyti bei organizuota bandomoji FKU molekulinių genetinių tyrimų IKĮ schema, kuri taps atitinkamos EMQN IKĮ schemos pagrindu.