Translational research in the post-genomic era: advances in the field of transcriptomics

Pesquisa translacional na era pós-genômica: avanços na área da transcriptômica

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DOI: 10.1590/0103-110420195213

ABSTRACT Translational research involves the interface between basic research and medical practice in order to generate innovative products or processes to introduce them into clinical protocols and health systems. The objective of this essay was to present an overview of transcriptomic advances, subsidized by the availability and use of new information technologies and molecular biology. In the search for accurate and less invasive diagnosis, transcriptomic tests use gene expression signatures to detect neurodegenerative diseases (Parkinson and Alzheimer), autoimmune (systemic lupus erythematosus, Wegener's granulomatosis), heart failure, autism and cancer (breast, colorectal, hepatic and lung). In the English health system the clinical guidelines incorporate eight transcriptomic tests, all with a focus on cancer. In Brazil genomic tests based on DNA sequences are regulated to diagnose congenital anomalies both in the Unified Health System and in supplementary health, but the molecular tests have not advanced in the scope of the diagnostic transcriptomics. The Brazilian health system should go beyond the tests of genomic analysis and begin the process of regulation of transcriptomic diagnostic technologies. In the future, diagnostic tests evaluating multiple gene expression profiles may become routine exams in a form of molecular screening.

KEYWORDS Translational medical research. Transcriptome. Diagnosis.

RESUMO *A pesquisa translacional envolve a interface entre a pesquisa básica e a clínica médica com o intuito de gerar produtos ou processos inovadores para introduzi-los nos protocolos clínicos e nos sistemas de saúde. O objetivo desse ensaio foi apresentar uma visão geral dos avanços da transcriptômica, subsidiados pela disponibilidade e utilização das novas tecnologias da informação e biologia molecular. Na busca pelo diagnóstico preciso e menos invasivo, testes transcriptômicos utilizam assinaturas de expressão gênica visando detectar doenças neurodegenerativas (Parkinson e Alzheimer), autoimunes (lúpus eritematoso sistêmico, granulomatose de Wegener), insuficiência cardíaca, autismo e câncer (de mama, colorretal, hepático e de pulmão). No sistema de saúde inglês as diretrizes clínicas incorporam oito testes transcriptômicos, todos com foco no câncer. No Brasil testes genômicos com base nas sequências de DNA são regulamentados para diagnosticar anomalias congênitas, tanto no Sistema Único de Saúde, como na saúde suplementar, mas os testes moleculares não avançaram no âmbito da transcriptômica diagnóstica. O sistema de saúde brasileiro deveria ir além dos testes de análise genômica e*

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iniciar o processo de regulamentação das tecnologias transcriptômicas de diagnóstico. No futuro, testes diagnósticos avaliando múltiplos perfis de expressão gênica podem se transformar em exames de rotina numa forma de triagem molecular.

PALAVRAS-CHAVE *Pesquisa médica translacional. Transcriptoma. Diagnóstico.*

Introduction

Translational research involves, at its earliest stage, technology transfer, where knowledge generated in the basic sciences leads to the production of new products, such as drugs, equipment, diagnostic tests and innovative treatment options. In this interface between basic research and medical clinic, the aim is the generation of an innovative product and its introduction in clinical protocols and health systems. Another phase of translational research encompasses the dissemination of innovations produced, ensuring that new technologies and knowledge generated in research reach the end user[1]. Originating from the 'bench to bedside' concept, translational medicine aims to eliminate barriers between research laboratories and clinical practice[2].

Within the scope of translational genomic (and post-genomic) research, the translation of scientific knowledge into advances in clinical practice still represents a challenge. Recently, scientists have focused on the application of human genomic knowledge in the health sector, in order to assist in the diagnosis and treatment of diseases. Returning to the beginning of genomic studies, the first sequencing of the human genome was carried out by an international consortium, cost millions of dollars and took more than a decade for the genome sketch to be published in 2001[4]. After the elucidation of the human genome sequence, the press and the public called for rapid responses, such as personalized medicine and the molecular diagnosis of genetic-based diseases (genetic examination of the individual for diagnostic purposes). Advances in human genomics generated high expectations and certain 'translational anxiety'[5]. Scientists wondered: how to get to the translation of basic biomedical knowledge into clinical practice?[6].

The transfer of technology based on this knowledge took some time because of the complexity of genetic information, but it is already becoming reality. Following the flow 'data → information → knowledge', researchers from all over the world have struggled to take the advances generated on the bench to the hospital bed and the health system, developing products accessible to the public. Information technology is an indispensable tool in translational medicine in relation to the economic sciences (genomics, transcriptomics, proteomics)[6], because the large volume of data makes the analysis practically impossible manual.

The post-genomic era began two decades ago, and since then sequencing equipment and techniques have evolved rapidly to lower the cost of analysis and dramatically decrease the time required for sequencing a complete genome. It is noteworthy that while genomic data are decisive for the understanding of pathologies and effects of drugs in physiological systems, the gap between genotype (individual genetic load) and phenotype (observable characteristics) can be studied by characterizing the different omic levels, including intermediate levels: transcriptome (RNA sequences and levels), proteome (the set of proteins in the

sample), metabolome (the set of metabolites)[7]. In addition to genome sequencing (the individual's DNA sequence), variations in methodologies allow further analysis, such as sequencing and quantification of transcripts (RNA) by the RNA-Seq[8] technique.

The huge amount of biological data generated in the last decade by large-scale transcriptomics studies deposited in public biological databases allows secondary studies to be conducted generating viable products that can be used in the molecular diagnosis of diseases. It is possible that certain physiological states can be characterized by gene expression signatures. These expression signatures are gene expression patterns, sets of genes linked to diseases that can be used as molecular diagnostic tests. Some recent developments in translational transcriptomics in several areas of medicine will be described below.

The objective of this study was to present an overview of the advances in the transcriptomics area subsidized by the availability and use of new information technologies and molecular biology. Based on the methodology called design science[10], transcriptomics studies were reviewed and examples of diagnostic tests based on gene expression patterns were presented. The fragilities of the transcriptomics studies were considered in the following section, followed by a description of technologies already incorporated and regulated.

Design science

As a way of designing the questions that guided this study, the design science model was used, which seeks to understand and identify the main points of the study, based on problem solving[10]. In this approach, there is the General Research Question (GRQ), which can be categorized in other more specific questions; for the present study, four Conceptual Questions (CQ) were considered. Therefore, the general question of research is:

- GRQ: how are new technologies for molecular diagnosis in the transcriptomics area being used and made available?

Thus, the above mentioned GRQ can be decomposed into the following conceptual research questions presented below:

- CQ 1: what are the means for molecular diagnosis in transcriptomics?

- CQ 1.1: what molecular diagnostic tests are in the development stage?

- CQ 1.2: what molecular diagnostic tests are regulated?

- CQ 1.3: which molecular diagnostic tests are incorporated and used in health systems similar to SUS?

In design science, the structuring of the research problem-set is carried out through the decomposition of the questions previously outlined, and the construction of the solution-set occurs by the composition of solutions of the questions. The solutions are presented throughout the work.

Methods of transcriptomics studies

Transcriptomics studies identify and quantify RNA in different tissues and in different physiological conditions. The most widely used techniques in transcriptomics studies are: RT-qPCR; qPCR array, microarrays and RNA-seq. The RT-qPCR or Quantitative Polymerase Chain Reaction with reverse transcription (Reverse transcriptase quantitative Polymerase Chain Reaction) evaluates gene expression on a punctual basis (gene by gene). The quantitative PCR arrangement (qPCR-array) uses the RT-qPCR to evaluate changes in the expression of tens to hundreds of genes. Microarrays use the hybridization of nucleic acids to evaluate gene expression. RNA-seq uses sequencing to quantify transcripts. All

these techniques (RT-qPCR, qPCR-arrays, microarrays and RNA-seq) present the results in fold-change (indicating how many times the RNA concentration has increased or decreased), and their data can be used in comparative studies, for example, analyzing changes in gene expression resulting from physical exercise[11].

The conventional PCR technique was developed in the 1980s based on the amplification of specific fragments of the DNA[12]. This technique is capable of detecting whether the sought fragment is present or missing in the sample but does not quantify the genetic material in the sample. The RT-PCR technique is presented as a variation of the conventional PCR in which the genetic material is labelled with a fluorescent reagent and the detection of this fluorescence is performed after each amplification cycle. Because of the fluorescence cycle detection, the RT-qPCR is able to quantify the genetic material in the sample in a comparative way, giving the result in fold-change, which indicates how often the RNA concentration is larger or smaller in a sample comparing to a control sample. RT-qPCR is a point analysis that evaluates gene by gene. The q-PCR array technique (quantitative PCR arrangement) is a RT-qPCR in which several genes are evaluated in parallel. Using q-PCR array, tens to hundreds of genes can have their expression levels evaluated at the same time.

The complementary DNA microarray technique is based on the hybridization of nucleic acids, being a system capable of detecting the expression of a large number of genes in parallel. Thousands of probes for the genes of interest are adhered to specific points on a solid support. In this technique, two samples of RNA (transformed into complementary DNA), marked with distinct fluorescence, are evaluated concomitantly (test vs. control). According to the fluorescence detected, the relative concentrations of transcripts in the samples can be measured. With the use of microarray, it is possible to evaluate complex gene expression patterns and develop sensors for use in clinical diagnoses[13].

RNA-seq is a modern technique of molecular biology that uses the deep sequencing of complementary DNA (produced from RNA) to quantify differential gene expression. After sequencing, elucidated sequences are mapped using the reference genome, and the assessment of the presence and quantity of each RNA can be calculated and compared to the quantities in another sequence sample. With the use of RNA-seq, it is possible to measure the presence and prevalence of known and previously unknown transcripts[8].

The immense amount of biological data generated in the last decade by large-scale transcriptomics studies deposited in public biological databases allows secondary studies to be conducted generating viable products that can be used in the molecular diagnosis of diseases. Some recent developments in translational transcriptomics in several areas of medicine will be described below.

Translational transcriptomics in the development of diagnostic tests

In the search for accurate diagnosis of complex diseases, transcriptome-based tests have been developed to detect various diseases. Among the diseases with molecular diagnosis based on gene expression are some neurodegenerative diseases[14,15], autoimmune[16,17], cardiomyopathies[18,19] and autism[20,21].

Molecular tests of transcriptome-based neurodegenerative diseases have been developed for Alzheimer's and Parkinson's. In 2014, the company Siemens Healthcare Diagnostics filed the patent for a diagnostic test for Alzheimer's disease, a disease whose early diagnosis poses a challenge, as the initial symptoms resemble other neurological disorders, as well as natural aging processes. The researchers used the RNA-Seq technique to analyze gene expression,

specifically microRNAs (miRNAs), and developed a blood tissue diagnostic test that evaluates the expression pattern of 10 miRNAs (molecular markers for Alzheimer's disease)[14].

MiRNA expression patterns can also be used for the detection, prognosis and monitoring of Parkinson's disease in blood, serum or skin samples. In a molecular approach based on gene expression, a set of 142 genes with disease-specific transcriptomics profile were revealed. The expression pattern of these miRNAs, when compared to healthy individuals, included 72 genes with an increased expression level and 70 genes presenting a lower expression in individuals with Parkinson's disease[15].

In 2006, the MetriGenic Corporation (Canada) patented transcriptomics patterns associated with autoimmune diseases, particularly: systemic lupus erythematosus, Wegener's granulomatosis, and anca-positive vasculitis. The tests included the analysis of the expression of a set of 1.645 genes or their subsets, being able to make the differential diagnosis of the autoimmune diseases mentioned. The test can also be used to classify diseases into subgroups and predict the presentation of symptoms of systemic lupus erythematosus[16].

Rheumatoid arthritis was reviewed by Burska and collaborators, in which methods of diagnosis, prognosis and prediction of response to gene expression-based therapies were evaluated. The described protocols included transcriptomics tests for the diagnosis of rheumatoid arthritis and osteoarthritis, as well as trials that distinguished between rheumatoid arthritis and osteoarthritis according to the gene expression pattern. Researchers evaluated several gene expression signatures, with generally inconclusive comparisons, and realized a great need to harmonize study methods and protocols for gene expression patterns to become diagnostic tools in medical clinic[17].

In the field of transcriptomics studies of cardiomyopathies, Liu and collaborators used RNA-Seq technology in the cardiac tissue of a group of six volunteers (three in the control group, one patient with ischemic heart disease and two with dilated cardiomyopathy) to define expression patterns aimed at detecting heart failure. Using the generated gene signature, the researchers tested over 313 samples of heart tissue and were able to classify heart failure appropriately[19].

A study sponsored by CardioDX (USA), involving more than 1.000 volunteers, evaluated the serum transcriptome of individuals to refine and validate an RT-PCR assay for coronary heart disease. The pattern of gene expression in patients' blood (non-diabetic) was evaluated using a set of 23 genes, and the results were compared to coronary angiography data. The developed test, called 'Corus CAD', takes into account the biological differences between genders, and is, therefore, gender-specific. Overall, the test had a sensitivity of 85% and specificity of 43%[18]. The 85% sensitivity was at a good level, but the 43% specificity indicated a high rate of false positive results and reflected a need for improved testing. The company was approved by the U.S. Food and Drug Administration (FDA), and 'Corus CAD' was listed on the list of exams offered by the American government's 'Medicare' health insurance, between 2012 and 2018. According to a report from the newspaper 'San Francisco Chronicle', Medicare terminated the coverage of the test 'Corus CAD' at the end of 2018, judging the unnecessary test and of little usability for patients, which led the company Cardio DX to close its doors in early 2019[22].

Hu[19] (2009) studied cell lines from monozygotic twin blood samples with different diagnoses in search of a molecular profile for the detection of autistic spectrum disorder. A microarray method for screening for autistic spectrum disorders was designed to evaluate the gene expression of the individual. The gene pool for diagnostic use

includes 25 more expressed genes and 19 genes with a lower level of expression in autistic individuals[20].

Another transcriptome-based test for the detection of autistic spectrum disorders has been patented by Kunkel et al[21]. The method of characterizing and diagnosing autistic spectrum disorders described in the patent can be used with brain, spinal fluid, or blood samples in a gene expression analysis system with the evaluation of at least 10 genes within a list of hundreds of genes presented, followed by the classification of the molecular phenotype from a classifier algorithm[21].

Given that there is a great effort towards the development of molecular tests for cancer diagnosis, these will be presented in a separate item.

Translational transcriptomics of cancer

The scientific literature, as well as the patent banks, revealed diagnostic tests developed to detect some types of cancer by analyzing transcriptomics patterns. Several tests are being developed, among them, some aimed at detecting breast, colorectal, hepatic and lung cancer. It is noteworthy that these generally evaluate tissues that can be collected in a less invasive way than the organ's own biopsy, for example, blood samples, cells of the nose[23-28].

Aarøe and colleagues evaluated the blood of breast cancer patients and compared it to healthy women using the microarray technique. The researchers identified a blood gene signature that classifies, with a good level of accuracy, individuals with and without breast cancer. The test produced includes probes to evaluate the expression of 738 genes. In breast cancer patients, the following expression signature was observed: 395 genes with higher expression levels and 343 genes with lower RNA concentration when compared to individuals without the disease[23]. A diagnostic test for breast cancer based on a blood sample transcriptome (rather than breast tissue biopsy) is much less invasive and can be used as a screening system to minimize health care spending.

Another in vitro method based on blood transcriptome changes to diagnose, identify and monitor breast cancer cases has been patented. The patented test includes detecting changes in the expression of a set of 345 genes or subsets of them when compared to a pattern of gene expression extracted from healthy subjects. The gene pool can be evaluated by transcriptomics analysis methods involving nucleic acid amplification (RT-PCR or qPCR arrays) or hybridization (microarray). Using this test, cases of breast cancer can be detected before other signs and symptoms become evident[24]. An early detection test contributes to lower mortality and lower health care costs as the transcriptome reveals the presence of a tumor before it can be detected by other methods (such as mammography) and treatment can be initiated before it progresses and becomes invasive.

In the search for the accurate diagnosis of colorectal cancer and other related diseases, Galamb and collaborators[25] used microarrays to develop a transcriptomics profile capable of evaluating the material collected during biopsies and differentiating between colorectal cancer, irritated neck syndrome, adenomas and hyperplastic polyps. In order to classify colorectal diseases as inflammatory, benign or malignant, the authors proposed the use of an expression pattern of 18 genes, using knowledge of molecular biology in the differential[25] diagnosis. Hauptman and collaborators[26] used computational means to reevaluate results from several gene expression studies in the search for a means to differentiate between benign and malignant adenomas. The expression pattern of a set of 16 genes (COL12A1, COL1A2, COL3A1, DCN, PLAU, SPARC, SPON2, SPP1, SULF1, FADS1, G0S2, EPHA4, KIAA1324, L1TD1, PCKS1 and

C11orf96) was proposed by the authors as a method to distinguish between the different types of adenoma, in order to better target the treatment of the patients[26].

Hepatocellular carcinoma tends to be diagnosed at advanced stages of the disease and usually has a poor prognosis. Xie and collaborators[27] designed a diagnostic model based on a transcriptomics pattern in peripheral blood that differentiates between healthy subjects and patients with early-stage hepatocellular carcinoma. The proposed expression pattern, which evaluates the RNA of nine genes (GPC3, HGF, ANXA1, FOS, SPAG9, HSPA1B, CXCR4, PFN1 and CALR), presented 96% sensitivity and 86% specificity for the detection of the disease in an early stage.

In search of a noninvasive diagnostic method to detect lung cancer, a Boston University group developed a test based on the nose cell transcriptome. The test involves sampling cells from the nasal epithelium and analysis of the gene expression of 535 genes or different subsets of them (with 20, 40, 60 or 70 genes). The expression pattern of these genes, when compared to the transcriptome of individuals without the disease, reveals whether the individual has lung cancer using a noninvasive collection procedure and a more accurate analysis methodology than the other tests available for diagnosis and prognosis (chest X-ray, bronchoscopy, sputum cytological analysis and tomography)[28]. However, some technical challenges still persist and will be explained below.

Challenges

Transcriptomics studies produced abundant data, but, so far, the comparison of gene sets generated in the different studies tends to be inconclusive, as in the case of studies with rheumatoid arthritis[17]. A major challenge in transcriptomics research is the reproducibility of the results, which makes it difficult to define a standard gene pool for the detection of a certain disease.

Molecular mechanisms of transcription (producing RNA) and translation (producing proteins) are key processes in disease etiology. Disease development is influenced by several environmental factors and depends on highly dynamic interactions in several layers: DNA, epigenetics (modifications in chromatin and DNA that alter gene expression), RNA, proteins and metabolites[29]. Factors that may influence analysis results include sample source, experimental methodologies, and analytical tools[17]. In fact, the transcriptome is quite dynamic, and can be altered by several factors. Differences in in vivo experimental design, such as the time of collection, the biological material collected, if the individual has eaten or is fasting at the time of collection, if the individual has exercised in the last 24 hours can affect the result of the analysis.

There is a need for harmonization of studies so that expression signatures linked to certain diseases can be elucidated, aiming at producing new biomarkers for use in clinical practice[17]. The preparation of the individuals for the collection of samples should be standardized in order to allow the comparison between the results of several studies in meta-analysis and the design of good sets of biomarkers genes. The need for standardization of transcriptomics studies goes beyond the in vivo stage (with human beings), with bench stages and data processing. The studies of RT-PCR and qPCR arrays compare the expression of target-genes with control-genes. The definition of standard control-genes to study certain diseases would facilitate the standardization and reproducibility of the studies. In the case of studies by microarrays and RNA-seq, the data treatment should be standardized, since different algorithms and different statistical thresholds in this processing lead to different gene sets.

Transcriptomics studies compare

individuals with the disease against healthy individuals. However, does the 'standard healthy individual' exist? We believe not. As previously mentioned, the transcriptome is highly dynamic, and several environmental factors influence it. The expression pattern of the control group of one study may differ from another by several aspects, both genetic and environmental (which include way of life, food, climate, pollution, stress, etc.). A solution to this bottleneck in transcriptomics research can be the comparison of two samples from the same individual before and after a given intervention. By explaining this line of thought, in a test to evaluate the transcriptome of diabetes, for example, a fasting blood sample could be taken, and after ingestion of a predetermined dose of glucose, wait a while under observation and take another sample. Comparison of 'after' versus 'before' glucose intake will reveal how your body reacted to glucose. The metabolism of diabetic or pre-diabetic individuals will react differently to the glucose dose when compared to non-diabetic individuals. In this way, we would be eliminating the need to establish a genetic profile for the 'standard healthy individual'.

Regulation of transcriptomics diagnostic methods

In the health system of England (National Health Service – NHS), the body responsible for advising and regulating the incorporation of health technologies is the National Institute for Health and Care Excellence (NICE). Searches in the NICE guidelines (www.nice.org.uk) with the expression 'gene expression' and the term 'RNA' revealed eight transcriptomics tests already regulated in England, all focusing on cancer. In the transcript evaluation of breast cancer, some tests were found evaluating the probability of recurrence of cancer in a ten-year period (EndoPredict, MammaPrint, Oncotype DX and Prosigna) and rapid tests to evaluate if there is metastasis in lymph node samples (RD-100i OSNA and Metasin). In the evaluation of prostate cancer, the PROGENSA PCA3 test evaluates prostate cells in urine samples for diagnosis and the Prolaris test evaluates the transcriptomics profile of tumor samples to predict the risk of mortality in ten years[30].

In Brazil, the National Commission for the Incorporation of Technology in the SUS (CONITEC) advises the Ministry of Health on the elaboration of clinical protocols and therapeutic guidelines and on the incorporation of health technologies by SUS[31]. A search of the CONITEC guidelines (with the terms 'gene expression' and 'RNA') did not reveal relevant results within the scope of diagnostic transcriptomics.

Genomic tests based on DNA sequences are already regulated in Brazil to diagnose congenital anomalies. The National Supplementary Health Agency (ANS) issued Technical Note 876/2013/GEAS/GGRAS/DIPRO/ANS with guidelines for the use of molecular DNA analysis procedures with about 30 genetic tests that should be available to health plan users[33]. However, genomic analyses only evaluate the individual's genetic load (DNA). Brazil, like England, should go beyond genomic analysis tests and begin the process of regulation of transcriptomics diagnostic technologies.

In this essay, we aimed to describe molecular diagnostic tests that were incorporated and used in health systems similar to SUS, which excludes the United States of America. Therefore, no Food and Drug Administration (FDA) data were evaluated.

Final considerations

The post-genomic era brought new challenges and opportunities for diagnostic medicine. In the transcriptomics area, bench researches have generated gene expression signatures linked to several diseases, with the need for translational research for the production of diagnostic tests,

and to ensure the transfer of technology and its application in health systems.

In the future, diagnostic tests evaluating multiple gene expression profiles may turn into routine examinations in a form of molecular screening, for example, for several types of cancer. A blood test may reveal if there is a higher probability of developing cancer in a given organ, and more specific (and more invasive) tests would then be performed to confirm the diagnosis. Molecular screening by transcriptomics methods can contribute to reducing mortality and saving resources for health systems by the ability to detect diseases early.

Collaborators

Pacheco C (0000-0003-1829-1515)*, Ceccatto VM (0000-0003-4839-4400)* and Maia CM (0000-0002-7540-7177)* contributed to the design and planning of the study; preparation of the first versions; critical review of the content; approval of the final version of the manuscript. Rosa SSRF (0000-0002-1247-9050)* contributed to the accountability for the whole work and approval of the final version. Leite CRM (0000-0003-1857-6238)* contributed to the accountability for the whole work, drafting of the manuscript and approval of the final version. ■

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References

1. Woolf SH. The meaning of translational research and why it matters. JAMA. 2008; 299(2):211-213.

2. Marincola FM. Translational medicine: a two-way road [internet]. J Transl Med. 2003 [acesso em 2019 mar 10]; 1:1. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC202357/.

3. Taylor N, Best S, Martyn M, et al. A transformative translational change programme to introduce genomics into healthcare: a complexity and implementation science study protocol [internet]. BMJ Open. 2019 [acesso em 2019 mar 10]; 9(3). Disponível em: https://bmjopen.bmj.com/content/9/3/e024681.abstract.

4. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome [internet]. Nature. 2001 [acesso em 2019 mar 10]; 409:860-921. Disponível em: https://www.nature.com/articles/35057062.

5. Khoury MJ, Gwinn M, Ioannidis JPA. The Emergence of Translational Epidemiology: From Scientific Discovery to Population Health Impact [internet]. American Journal of Epidemiology. 2010 [acesso em 2019 mar 10]; 172:517-524. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2927741/.

6. Liu L, Li Z. Bridging the basic biomedical researches and clinical practices with biomedical informatics [internet]. Journal of Translational Medicine. 2012 [acesso em 2019 mar 10]; 10:A52. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3480033/.

7. Krzyszczyk P, Acevedo A, Davidoff EJ, et al. The growing role of precision and personalized medicine for cancer treatment [internet]. Technology. 2018 [acesso em 2019 março. 10]; 6(3-4):79-100. Disponível em: https://www.worldscientific.com/doi/10.1142/S2339547818300020.

8. Mortazavi A, Williams BA, McCue K, et al. Mapping and quantifying mammalian transcriptomes by RNA-Seq [internet]. Nature Methods. 2008. [acesso em 2019 mar 10]; 5(7):621-8. Disponível em: https://www.nature.com/articles/nmeth.1226.

9. Moreira CO, Ferraz ASM, Carvalho DP, et al., inventores; Universidade Estadual do Ceará, titular. Kit para análise de fenótipo de performance física, processo para determinação de fenótipo de performance física e uso do kit. Brasil n.PI 1020180058509, 2018 Mar 23.

10. Wieringa RJ. Design science methodology for information systems and software engineering. Berlin: Springer; 2014.

11. Pacheco C, Felipe SMDS, Soares MMDC, et al. A compendium of physical exercise-related human genes: an 'omic scale analysis [internet]. Biol Sport. 2018 [acesso em 2019 mar 10]; 35(1):3-11. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6135974/.

12. Mullis, KB, Faloona, FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction [internet]. Methods Enzymol. 1987 [acesso em 2019 mar 10]; 155:335-350. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/3431465.

13. Schena M, Shalon D, Davis RW, et al. Quantitative monitoring of gene expression patterns with a complimentary DNA microarray. Science. 1995; 270:467-470.

14. Keller A, Stahler CF, Meese E, et al., inventores. Siemens Aktiengesellschaf, titular. Diagnostic miRNA markers for Alzheimer. World Patent WO 2014075911A1. 22 Maio 2014.

15. Youdim MBH, Mandel SA, Grunblatt E, et al., inventores. Technion Research and Development Foundation Ltd, titular. Diagnostic test for Parkinson's disease. United States Patent US 2010/0221735 A1. 2 Set 2010.

16. Barnes DA, Dempsey A, inventores. Metrigenix Corporation, Debra A. Barnes, Adam Dempsey, titulares. Markers for autoimmune disease detection. World Patent WO 2006020899A2. 23 Fev 2006.

17. Burska AN, Roget K, Blits M, et al. Gene expression analysis in RA: towards personalized medicine [internet]. Pharmacogenomics J. 2014 [acesso em 2019 mar 10]; 14(2):93-106. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/24589910.

18. Rosenberg S, Elashoff MR, Beineke P, et al. Multicenter Validation of the Diagnostic Accuracy of a Blood-Based Gene Expression Test for Assessing Obstructive Coronary Artery Disease in Nondiabetic Patients [internet]. Ann Intern Med. 2010 [acesso em 2019 mar 10]; 153(7):425-434. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/20921541.

19. Liu Y, Morley M, Brandimarto J, et al. RNA-Seq Identifies Novel Myocardial Gene Expression Signatures of Heart Failure [internet]. Genomics. 2015 [acesso em 2019 mar 10]; 105(2):83-89. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/25528681.

20. Hu VW, inventor. The George Washington University, titular. Method and kit for diagnosing Autism using gene expression profiling. United States Patent US 20090117562A1. 7 Maio 2009.

21. Kunkel LM, Kohane IS, Kong SW, et al., inventores. Children's Medical Center Corporation, titular. Methods and compositions for characterizing autism spectrum disorder based on gene expression patterns. World Patent WO 2013066972A1. 10 Maio 2013.

22. Kunthara S, Ho C. CardioDx, maker of heart disease test, shutting down as Medicare rescinds coverage [internet]. San Francisco Chronicle. 2019. [acesso em 2019 jan 9]. Disponível em: https://www.sfchronicle.com/business/article/CardioDx-maker-of-heart-disease-test-shutting-13518778.php.

23. Aarøe J, Lindahl T, Dumeaux V, et al. Gene expression profiling of peripheral blood cells for early detection of breast cancer [internet]. Breast Cancer Res. 2010 [acesso em 2019 mar 10];12(1):R7. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/20078854.

24. Dumeaux V, Lund E, inventores. University of Tromsoe, titular. Gene expression profiles in diagnostics. World Patent WO 2014081313A1. 30 Maio 2014.

25. Galamb O, Sipos F, Solymosi N, et al. Diagnostic mRNA Expression Patterns of Inflamed, Benign, and Malignant Colorectal Biopsy Specimen and their Correlation with Peripheral Blood Results [internet]. Cancer Epidemiol Biomarkers Prev. 2008 [acesso em 2019 mar 10]; 17(10):2835-45. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/18843029.

26. Hauptman N, Boštjančič E, Žlajpah M, et al. Bioinformatics Analysis Reveals Most Prominent Gene Candidates to Distinguish Colorectal Adenoma from Adenocarcinoma [internet]. Biomed Res Int. 2018. [acesso em 2019 mar 10]. Disponível em: https://www.hindawi.com/journals/bmri/2018/9416515/.

27. Xie H, Xue YQ, Liu P, et al. Multi-parameter gene expression profiling of peripheral blood for early detection of hepatocellular carcinoma [internet]. World J Gastroenterol. 2018 [acesso em 2019 mar10]; 24(3):371-378. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5776398/.

28. Brody JS, Spira A, Berman J, et al., inventores. The Trustees of Boston University, titular. Diagnostic and prognostic methods for lung disorders using gene expression profiles from nose epithelial cells. World Patent WO 2007103541A2. 8 Mar 2007.

29. Sun YV, Hu YJ. Integrative Analysis of Multi-omics Data for Discovery and Functional Studies of Complex Human Diseases [internet]. Adv Genet. 2016 [acesso em 2019 mar 10]; 93:147-190. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26915271.

30. National Institute for Health and Care Excellence (NICE) [internet]. 2019 [acesso em 2019 mar10]. Disponível em: https://www.nice.org.uk/.

31. Comissão Nacional de Incorporação de Tecnologias

no SUS (CONITEC) [internet]. 2019. [acesso em 2019 mar 10]. Disponível em: http://conitec.gov.br/.

32. Comissão Nacional de Incorporação de Tecnologias no SUS (CONITEC): Recomendações sobre as tecnologias avaliadas. 2014. [acesso em 2019 mar 10]. Disponível em: http://conitec.gov.br/decisoes-sobre-a-incorporacao-de-tecnologias-no-sus-2014.

33. Agência Nacional de Saúde Suplementar (ANS). Nota Técnica 876/2013/GEAS/GGRAS/DIPRO/ANS. Rio de Janeiro: Agência Nacional de Saúde Suplementar; 2013.

Received on 04/16/2019
Approved on 09/10/2019
Conflict of interests: non-existent
Financial support: the project had the financial support of the Coordination for the Improvement of Higher Education Personnel (Capes).