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Letter to the Editor

Evaluation of genotyping methods and costs for VDR polymorphisms

Dear Editor,

We read with great awareness the work published by Di Spigna G et al[1]. The authors affirmed that the genetic screening Vitamin D Receptor (VDR) polymorphisms could be a useful tool for early identification of the osteoporosis in women patients with rheumatoid arthritis (RA). In this work, the authors have used a commercial kit based on Restriction Fragment Length Polymorphism (RFLP) method for VDR polymorphisms detection in RA patients. They conclude with the important issue "the clinician and the lab manager may join to evaluate costs and availability, of the appropriate methods to setting molecular diagnostics of VDR Genotyping". We agree to this affirmation.

In general, as genomics tests performed widely in clinical laboratories, the evaluation of the best commercially available platforms becomes a noteworthy consideration about the clinical employment of genetic information. Nowadays, genetic tests are performed either by the academic ultra-specialized lab or custom service laboratories that using certified commercial kits (when available). In Europe, the field of diagnostic products is regulated by "in vitro Diagnostic" (IVD) policy, without a distinction between diagnostics service and commercial products. In both cases, clinical laboratories may develop tests in-house ("home-brew") and validated them by submitting standardized results to outside referenced laboratories in the context of International External Quality Assurance (EQA) programs[2,3].

Payment and reimbursement for genetic testing are another issue of enormous importance that is already creating controversy among health care providers and join between patients and health insurance companies. It will be motivating to see whether insurers will consider genetic testing to be cost-effective. However, if the detection of VDR polymorphisms is routinely incorporating into clinical practice, knowledge concerning the predictive value of test which will eventually enable of individual therapy (Table I)[4].

Some methods to assess the quality and cost-effectiveness of genetic tests have now available. Noteworthy is the authoritative Diagnostic Advisory Committee of National Institute for Health and Clinical Excellence (NICE) which stimulates Health Company and governance communities to create data for fitting economic models into healthcare systems[5].

Current Genotyping Methods

The qualitative assessments of the VDR Single Nucleotide Polymorphisms (SNPs) could perform without an underlying gold standard method for the daily diagnostic routine (Table II).

Technological platforms most widely used for the genotyping of known SNPs include: I) PCR-based methods without fluorescent emission as Allele Specific Amplification and RFLP; II) PCR with Fluorescent hybridization probes as FRET-based platforms, Locked Nucleic Acid Probes and Invader assay; III) PCR-based with intercalating fluorescent dye as High-Resolution Melting; IV) pre-treatment PCR only, as Denaturing-High Performance Liquid Chromatography; and V) sequencing methods either as automated Sanger's sequencing or high-throughput sequencing technologies called "Next Generation Sequencing" (NGS).

Genotyping Costs

The primary intention of a cost-effectiveness analysis is to provide adequate information for decision-makers to distribute funds in the genetic tests for the healthcare improvements. Overviews of cost-effectiveness studies on genetic assay and platforms in healthcare fields are now available[6].

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Table I.

SNP code	Genetic variant	MAF*	Clinical annotation	Genetic annotation
rs2228570	FokI T>C (Met1Pro)	0.35 T	Genotype TT (called ff) is correlated to low BMD in woman	Shorter protein (loss 3 aa) in TT phenotype
rs1544410	BsmI Intron 8 A>G	0.26 A	Genotype AA (called BB) correlating to lower BMD, because of an intestinal calcium uptake reduction.	Loss of protein stability and quantity with BB phenotype
rs731236	TaqI Exon 9 nucleotide 352T>C	0.26 C	Genotype TT (called TT) is associated to low BMD in a woman with osteoporosis.	Lower affinity binding to Vitamin D with TT phenotype
rs7975232	ApaI T>G	0.50 T	Genotype TT (called AA) synergizing effect of TaqI TT allele	Lower affinity binding to Vitamin D with AA phenotype

*Minor Allele Frequence.
Abbreviations: BMD, *Body Mass Density, aa aminoacid*.

Table II. Current platforms for detection VDR polymorphisms.

Genotyping methods to detect known SNP	Instrument mean costs§	Reagent costs per SNP$	Approximate time-labour per SNP#
Allele Specific Amplification (ASA)	+	Very low	Moderate
Restriction Fragment Length Polymorphism (RFLP)	+	Very low	Very laborious
FRET probe Allelic Discrimination (Hyb Probe® TaqMan®, Beacons® Scorpions®)	++	Moderate	Moderate
High resolution melting (HRM)	++	Low	Moderate
Conventional Sanger sequencing (automated with fluorescent detection)	++	Low	Moderate
Next Generation sequencing (NGS)	++++	Very high	Very fast
Denaturing-High Performance Liquid Chromatography (D-HPLC)	++++	Moderate	Very fast

§Approximate instrumentation list price were scored as + (<10000€); ++ (<50000€); +++ (<100000€), ++++ (>100000€)
$Reagent costs scored as very low (<5€), low (<10€), cheap (<30€), high (<50€), very high (>50€).
#Time-labour refers input needed to perform a single test of multiple samples. It were scored as very fast (< 1 hour), fast (<4 hours), moderate (< 1 day), laborious (<2 days) very laborious (>2 working day).

However, the literature is still low of studies addressing the economic implication in clinical healthcare of genomics tests. Significant study to compare the cost of two methodologies validated for genotyping variations in the cytochrome P450 subtype 2C9 gene: the cost/sample for single SNP detection was $1.90 (US dollars) by PCR-Pyrosequencing and $ 3.14 by RFLP[7]. In this case, the instrumentation cost is averaged $100,000 and $5,000 respectively. It is clear that the better platforms are directly correlating to a number of samples. Furthermore, when the number of processing sample is little, genotyping cost should be dramatically reduced by "home brew" validated tests. For example, an early outline of pharmacogenomic tests performed on FRET-Assay platforms averaging about $20 per SNP[8,9].

The initial framework evaluation costs of the detection of VDR gene variants could average about $5 per polymorphisms by RFLP platform (Table II).

Conclusions and Future Outlook

We still need precise evidence that genetic tests offer an added value, regarding relative cost and benefit. Also, there is a more genomic expertise to interpret the results of the tests efficiently[10-11].

The usefulness of the genetic markers in clinical practice depends on improving the diagnostic prediction or endorsement ameliorative treatments strategy[12]. There is an undeniable need for more detailed and extensive studies to establish the cost and effectiveness of genotyping. With new genetic markers being identified and validated, physicians will have new ways and means to tailor specific therapy to individual genetic profiles[13].

Consequently, it is crucial that pharmaceutical and biotechnology companies join their future investments to develop accurate and low-cost genetics tests for routine diagnostics. Promising, decision-maker might be able to accelerate the translation of genetic technologies into the routine clinical laboratory.

Conflict of interest

The authors declare no conflicts of interest.

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