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PREDICTIVE AND PERSONALIZED APPROACHES TOWARDS RETINITIS PIGMENTOSA AND CEREBRAL CAVERNOUS MALFORMATIONS

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Abstract

The most of chronic and common pathologies represent the result of intricate and heterogeneous causes, from heritable components to environmental elements. This complex picture represents a strong challenge towards the acknowledgement of diseases etiology, which could be fight by discovery and use of disease predisposing alleles. This purpose could be realized using many genetic tests, which could facilitate early treatment, preemptive selection of efficacious drugs, and more accurate estimation of risk, because severity and response to cure reflects the underlying individual allelic picture. But, if the effective advantages of such model are relevant for monogenic disorders, more complex results the situation for polygenic ones, as Retinitis pigmentosa and Cerebral Cavernous Malformations. Moreover, elements like lifestyle and environment, risk of false positive or negative, and accessibility to analysis data make the results and risks determined by predictive medicine more difficult to quantify. Finally, prediction could represent the future of translational research.

Keywords: Polymorphisms, NGS, Prediction Models.

Introduction and background

Today it is known that the most of chronic and common pathologies represent the result of intricate and heterogeneous causes [1]. They are the pieces of a puzzle made of heritable components such as DNA variants, methylation patterns, epigenetic RNA effects, and environmental elements such as chemical and physical agents exposure, or infection [2-5]. This complex picture represents a strong challenge towards the acknowledgement of diseases etiology, which could be fight by discovery and use of disease predisposing alleles [6]. Modern innovations delivered by Next Generation Sequencing permitted us a deep analysis into DNA variants and RNA expression changes, giving a more relevant role to genetic variations in understanding human diseases and drug response. The two most important areas benefiting of this genetic consciousness deal with the pharmacogenetics response, disease severity/follow up, and prediction of disease susceptibility [7, 8], as well as drug development deriving from the identification of molecular targets [9]. Nowadays only a few pharmaceuticals born directly from genetic findings serving as drug targets, but the list is expanding, targeting to specific biochemical pathways in order to improve clinical treatment, also reducing adverse reactions [10]. The use of genetic tests to disease prediction and diagnosis sees several applications, such as karyotyping for chromosomal abnormalities and, as realized in our laboratories, the enhancement of disease risk profiles using single nucleotide polymorphisms (SNPs) previously found to be disease-susceptibility markers [11]. Examples are given by BRCA1

and BRCA2 variants, that increase risk to breast and ovarian cancers [12], or by RP1 ones, involved into retinitis pigmentosa etiopathogenesis [13]. Clinical genetics testing can provide additional tools to accelerate and improve diagnosis and medical care. In particular, a relevant component of variation in disease course, severity, and response to cure reflects the underlying allelic picture existing in each individual, offering the opportunity to facilitate early treatment, preemptive selection of efficacious drugs, and more accurate estimation of risk for those considered to be at intermediate risk using traditional factors. This scenario could open the doors towards a personalized medicine, able to target individualized therapies and improve patients' healthcare [14]. Discovering of susceptibility variants represents a solid approach to investigate biochemical pathways that regulate diseases, especially for coding sequence or well-known regulatory variants, the most of which yet validated by functional studies [15]. Despite this, many causative or associated variants fall in intergenic and intronic regions, most of which not full functionally characterized yet [16]. Discovered genes and relative pathways they are involved in could represent pharmacological targets, such as realized for treatment of cystic fibrosis and hypercholesterolemia [17]. The help of bioinformatics models was fundamental to start a walkthrough towards following experiments that have establish the final involvement of genes into diseases ethiopathology [18]ook stationery_ Microsoft Excel preferences_ Microsoft Excel 97-2004 workbook_ Microsoft Excel XML spreadsheet_ Microsoft /. The most interesting and challenging application of genetic susceptibility data deals with the accurate prognosis of diseases (e.g., Mendelian and oligogenetic disorders, such as Stargardt disease or Cerebral Cavernous Malformations), or other fields involving medical decisions, such as choice of drug, selection of dose, avoidance of side-effects [19]. Unlike identifying potential drug targets, genetic-based prediction models could favorite the precise identification of molecular mechanisms and functional domains that drive genetic association/linkage signals [20]. The most sensible aspect making useful such analysis is represented by strength and robustness of the correlation across the population we considered. Because clinical decisions are personalized to each patient, physicians establish the probability of medical traits for each of them. This is not a static process, so physicians should update assessments as additional relevant information, such as laboratory tests (genetic or not), or changes in physiology become known. In this way, clinical decisions are interpreted as variation in an individual's risk to disease, severity of disease and response to medication are progressively revealed [21]. Summarizing, the ideal genetic-based predictive model for clinical applications should: (1) increase the posterior probability of medical traits compared to data obtained from existing known clinical tests-enough to enable changes in medical decisions and patient management, and (2) involve a relevant number of individuals to whom it is applied, improving resulting outcomes. Secondary, although not irrelevant, aspects regard cost-effective care and the ease realization and application of diagnostic tests. One of the most useful genetic informations, helping the development of such predictive models, come from genome-wide association study (GWAS) [22]. This kind of analysis could give us the number of loci, as well as frequency and penetrance of predisposing alleles, increasing the probability of identifying causal markers and the clinical utility of using those markers in affected population. But, if the effective advantages of such model are relevant for monogenic disorders, as Huntington's Chorea, more complex results the situation for polygenic ones, as Retinitis pigmentosa [23]. The difficulty is due to: 1) extremely various modes of inheritance (e.g., a few hundreds to thousands of loci and weakly penetrant alleles); 2) high locus/allelic heterogeneity (highly penetrant but unique loci and alleles involved across individuals); 3) high levels of epistasis; 4) ubiquitous epigenetic effects (such as histone acetylation or methylation patterns); 5) gene-environment interactions, or several combinations directly impacting the identification of predictive markers as well as their utility. So, whether GWAS analyze the common allelic architecture to find disease predisposing markers showing low degrees of allelic and locus heterogeneity, sequencing-based studies in families represent the first choice to discover rare disease-associated variants [24].

Available types of predictive medicine

- Newborn screening [25]: it is conducted just after birth in order identify genetic disorders that can be treated early in life. This testing of newborns for specific disorders is one of the most widespread uses of genetic screening, and ex-

amples are given by tests for homocystinuria, phenylketonuria, mucopolysaccharidosis and congenital hypothyroidism.

- Predictive Risk Testing [26]: it is conducted to determine the probability of developing a specific disease, over a given period of time. An example is the predictive risk test for hemochromatosis.
- Diagnostic testing [27]: it is often realized to confirm a particular diagnosis when a specific condition is suspected based on the subject's clinical symptoms. This type of tests ranges from blood pressure measurement and urine tests, to more invasive protocols such as biopsies, or to genetic screening.
- Medical bioinformatics [28]: this area involves the analysis of individual cell molecular parameters by cytomics and single cell-based microarrays. Relevant predictive medicine parameters would be extracted by computer-assisted identification and characterization of a little cell populations or gene clusters of interest (e.g., pathway analyses).
- Prenatal testing [29]: it permits to look for diseases and conditions in a fetus or embryo before it is born. This type of analysis is offered to couples who have an increased risk of having a baby carrying a genetic or chromosomal disorder. Like diagnostic testing, prenatal testing can be noninvasive (such as ultrasonography or maternal serum screening) or invasive (e.g., chorionic villus sampling).
- Carrier testing [30]: applied to identify people who carry one copy of a gene mutation that, when present in homozygosity, causes a genetic disease. It is mainly targeted to individuals with genetic disorder in their family history or to people in ethnic groups with increased risk of specific genetic pathologies. If both parents are tested, the exam can provide information about a couple's risk of having a child affected by a genetic disorder.
- Preconception testing [31]: it is conducted on two potential parents before a child is even conceived. This allows prospective parents to know the risk of diseases and likelihood of traits in their future offspring.

Examples: retinitis pigmentosa (rp) and cerebral cavernous malformations (ccms)

Cerebral cavernous malformations (CCMs) consist of enlarged capillaries characterized by impairment of intercellular junctions and cell extracellular matrix (ECM) adhesions and by the absence of pericytes [32]. The abnormally dilated capillary endothelial channels increases permeability, predisposing these vessels to episodes of thrombosis and focal hemorrhage, resulting in seizures and neurologic deficits [33]. The three CCM proteins coded by KRIT1 (CCM1), CCM2, and PDCD10 (CCM3) form a trimeric protein complex. Germline loss-of-function mutations in any of these genes may lead to the formation of CCMs [34]. Therefore, a common molecular pathway requires all three proteins to function together correctly for physiological cellular function [35]. Furthermore, several studies demonstrated that each component protein is able to interact with numerous other signaling and cytoskeletal molecules, allowing a wider range of functions in molecular signaling pathways via unique protein–protein interactions [36]. However, less than 70% of patients are clinically manifest, and a great percent of symptomatic ones represents sporadic forms. Developing an adequate genetic predictive test could help us to detect more "asymptomatic" cases, as well as a possible fourth causative gene representing the possible cause of yet unknown forms [37].

Retinitis pigmentosa (RP) represents a very heterogeneous inherited ocular disorder characterized by progressive retinal degeneration [38]. It involves retinal pigment epithelium and the photoreceptors (PRs), leading to a slow and progressive death in these cells, with the final impairment of visual neurotransmission [39]. The most frequent symptoms consist of night – blindness, tunnel vision, with involvement of both rods and cones. Instrumental exams usually highlight characteristic abnormal areas of pigment into the retina (usually during the advanced states of the disease), pale optic disc, and shrinked retinal vessels [40]. Today it is known that at least 50 genes, all involved into phototransduction and related pathways, are causative of retinitis pigmentosa different forms [41]. Mutations in these genes may be inherited in an autosomal recessive (50-60%), dominant (30-40%) [42]; [43] or X-linked (5-20%) [44] pattern, but about 30% is represented by sporadic form. However, today the greatest challenge is represented by the identification of many other unknown genes potentially causative of many still unidentified forms of retinitis pigmentosa [45]. Genetic predictive tests, using Next Generation Sequencing (NGS), could help us to find new disease-causing or associated genes, as well as strong genotype-phenotype correlations,

leading to a relevant enhancement of our understanding of allele pathogenicity, protein function and population genetics [46].

Limitations of predictive medicine

In many diseases, carrying the potential causative mutated gene still does not necessarily imply someone will get the disease. One aspect regards the protein level, which structure is less conserved than sequence [47]. Moreover, many complex and common diseases throughout the whole population are determined not only by hereditary, but also by lifestyle and environment (e.g., pollution, infection, physical exercises, diet, smoking) [48]. These elements make the results and risks determined by predictive medicine more difficult to quantify. Moreover, the potential false negatives or false positive that may come from a predictive genetic screen can cause substantial unnecessary tension on the individual [49]. Another question could deal with targeting medication to people who are genetically susceptible to a particular disease but do not yet show the symptomatology [50]. On one hand, many treatments carry undesirable side effects that high risk individuals must face. On the other, many populations-based prevention measures (such as healthy diets) carry a far lower likelihood of adverse effects and are also less expensive. Finally, potential problems of commercially available genetic testing could arise from the psychological impacts of accessibility to such data [27]. For single-gene inherited diseases, counseling and the right to refuse a test have been found to be relevant. However, correct individual counseling can be difficult to extend to the population likely to be identified as at high risk of common complex disease.

Conclusions

We have summarized the main issues in utilizing genetic information in predictive models for disease traits. Currently, the real challenge regards complex diseases, such as retinitis pigmentosa, and we suppose that several points could help to win: 1) The analysis of epigenetic modifications [51]; 2) Extension of predictive models to the widest different clinical populations [52]; 3) A more intensive application of NGS [53]; 4) Use of machine learning approaches to genetic data develop more powerful and accurate prognostic tests [54]; 5) Reduce disease complexity and improve genetic susceptibility by classifying diseases by molecular subtypes [55]. Finally, even if prediction will continue to be challenging, it could give researchers and clinicians a relevant help to discover new possible models to detect the real ethiopathology of many complex genetic and not diseases.

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