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Review

Systems medicine: the future of medical genomics and healthcare

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Published: 20 January 2009

Genome Medicine 2009, 1:2 (doi:10.1186/gm2)

The electronic version of this article is the complete one and can be found online at http://genomemedicine.com/content/1/1/2

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Abstract

High-throughput technologies for DNA sequencing and for analyses of transcriptomes, proteomes and metabolomes have provided the foundations for deciphering the structure, variation and function of the human genome and relating them to health and disease states. The increased efficiency of DNA sequencing opens up the possibility of analyzing a large number of individual genomes and transcriptomes, and complete reference proteomes and metabolomes are within reach using powerful analytical techniques based on chromatography, mass spectrometry and nuclear magnetic resonance. Computational and mathematical tools have enabled the development of systems approaches for deciphering the functional and regulatory networks underlying the behavior of complex biological systems. Further conceptual and methodological developments of these tools are needed for the integration of various data types across the multiple levels of organization and time frames that are characteristic of human development, physiology and disease. Medical genomics has attempted to overcome the initial limitations of genome-wide association studies and has identified a limited number of susceptibility loci for many complex and common diseases. Iterative systems approaches are starting to provide deeper insights into the mechanisms of human diseases, and to facilitate the development of better diagnostic and prognostic biomarkers for cancer and many other diseases. Systems approaches will transform the way drugs are developed through academy-industry partnerships that will target multiple components of networks and pathways perturbed in diseases. They will enable medicine to become predictive, personalized, preventive and participatory, and, in the process, concepts and methods from Western and oriental cultures can be combined. We recommend that systems medicine should be developed through an international network of systems biology and medicine centers dedicated to inter-disciplinary training and education, to help reduce the gap in healthcare between developed and developing countries.

Systems biology is developing rapidly. It is an integrative research strategy designed to tackle the complexity of biological systems and their behavior at all levels of organization (from molecules, cells and organs to organisms and ecosystems) in normal and perturbed conditions. It is based on an understanding of biological functions as system properties that are different from those of the individual interacting components (reviewed in [1-9]). It integrates the mass of data that has been collected with various global measurement technologies (techniques that look at the complete set of genes, proteins or other features in an organism), in order to formulate predictive mathematical and computational models of functional and regulatory biological networks. Specific biological hypotheses can thus be tested by designing a series of perturbation experiments. It thus combines data-driven (bottom-up) [10] and model-driven (top-down) [11] approaches into a question-driven (middle-out) inquiry in search of basic principles [12-14]. In the end,

systems approaches must be driven by high-quality hypothesis-driven biology and not just by data-accumulating technologies or high-performance computational modeling.

Although systems biology has ancient roots in physiology, biochemistry, and molecular and cellular biology, its current development is the result of recent advances in genomics and bioinformatics, which were made possible by the continuous development of high-throughput experimental and computational platforms. The field is also revisiting previous attempts at modeling biological complexity, by taking advantage of insights from system theory [15] and engineering sciences [16,17]. It forms the basis for an extension of genetic engineering into synthetic biology: designing and building biological systems with new properties from modular components [18-21].

Evolution, development, physiology and disease are viewed in systems biology as dynamic processes that operate on widely different scales in space and time between biological states that are constrained by interrelationships among pathway and network components. In this context, detecting, understanding and treating disease translates into identifying and manipulating global perturbed networks rather than focusing only on unique failing components.

Here we review how medical genomics [22,23], based on recent advances in high-throughput experimental and computational technologies, is evolving in the context of systems biology into a more prospective systems medicine [24-31]. This new kind of medicine will be able to overcome the current limitations of disease complexity (through stratification of patients and diseases by molecular diagnostics) and drug discovery (through the analysis and targeting of disease-perturbed networks) [32-35]. We discuss some of the technological, conceptual and organizational challenges that we will face in implementing this new vision and practice of biology and medicine, and we argue that it offers new opportunities to more efficiently tackle key medical problems in both developed and developing countries.

Technology is moving genomics from structure to function

The initial sequencing of the human genome was made possible by the automation of the DNA sequencing chemistry and by the development of data-acquisition tools and software for the reliable interpretation and assembly of the DNA sequence. It required a multi-billion-dollar investment and the participation of thousands of researchers in the public and private sectors over more than a decade. It came together with the sequencing of genomes in a variety of microorganisms, animals and plants. All these efforts combined served as test cases to trigger sustained technology developments.

With the next-generation DNA mega-sequencing technologies currently available, which enable the collection of billions of nucleotides in single instrument runs [36], it is now possible to sequence and assemble a human genome in a matter of weeks at a small fraction of the cost of the reference genome [37]. With both incremental progress and the introduction of third-generation sequencing technologies, it may soon become possible to collect large numbers of individual genomes in days for US$1,000 or less and ascertain their unique variations. This opens up the possibility of a Personal Genome Project to find correlations between genotypes and normal or diseased phenotypes [38]. In parallel, over a period of more than 30 years, successive generations of increasingly miniaturized DNA arrays have been used for expression profiling, benefiting from the extensive sequencing of partial and complete cDNA collections. Microarray technology, because of its intrinsic complexity and that of the transcriptome, has reached an intermediate stage of maturity compared with sequencing [39]; it is possible to detect variations in expression of many but not all gene transcripts under normal and perturbed conditions.

Early on, insufficient attention was paid by users of current microarray platforms to proper design and quality assessment, which is needed to control for variation in the large number of biological and experimental parameters involved. This compromised the usefulness of these platforms, for example in the development of classification and predictive biomarkers [40-42]. The introduction of standards and guidelines for complete microarray workflows [43] is helping to rectify the problem; these need to cover all aspects, from RNA integrity assessment [44,45] to data analysis and reporting [46,47]. There has also been constant progress in the use of advanced statistical methods for multivariate classification [48] and for gene-set enrichment analysis [49] of expression profiles. At the same time, it has become clear through the combination of tiling arrays and systematic sequencing that a larger fraction of the human genome is transcribed into diverse types of RNA than was previously thought [50,51]. The increased power and reduced cost of deep sequencing thus means that it is starting to compete with high-density microarrays [52,53], to the extent that some believe this is marking the beginning of the end of microarrays [54].

However, given that each new generation of tools takes several years to mature, it is most likely that sequencing and microarrays will continue to coexist. Microarrays will probably be increasingly used for specialized applications, such as those related to transcription regulation, epigenetic modifications, and selection of subfractions of individual genomes for sequencing (for example, exons, highly conserved regions, and so on); whereas mega-sequencing will be used for deep exploration of transcriptomes. The results of transcriptome analyses will increasingly be validated by emerging technologies that

use miniaturized high-throughput reverse-transcriptase PCR [55] or multiplex direct visualization and counting of RNA molecules; the latter technology has the added advantage of avoiding biases resulting from reverse transcription [56].

From the genome and transcriptome sequences, it has been possible to derive a relatively complete parts list of genes and, by extension, of proteins, thus revolutionizing the field of proteomics. It is crucial to note that mass spectrometry is effective in the identification of peptides, and not of complete proteins. In order to identify and quantify interesting proteins by mass spectrometry, either through shotgun or directed approaches, an investigator therefore needs to know the sequence of the peptides obtained by enzymatic digestion of those proteins. The current generation of proteomic tools that are based on high-performance combinations of chromatography and mass spectrometry thus enable the identification of a growing number of proteins, and can also identify them over a wide range of abundances and when they have complex secondary modifications. The technology can achieve this using fragmentation, peptide sequencing and, as noted above, comparison with proteins that have been predicted from genome and transcriptome sequences [57-59].

Recent results indicate that the description of complete reference proteomes is now within reach in advanced centers, using multiple reaction monitoring combined with mass spectrometry; this combination is the most powerful and rapid targeted approach currently available [60-62]. These complete proteomes will probably serve as a reference for the subsequent development of simpler targeted assays [63], which will be complemented by array-based global surveys using affinity-based protein-specific reagents [64-66], and in certain cases, by single-cell proteomics using high-speed flow cytometry [67,68]. Furthermore, ongoing developments using nanomaterials are expected to provide next-generation proteomic analysis tools [69].

In addition to using chromatography and mass spectrometry, metabolomics is also taking advantage of nuclear magnetic resonance to analyze complex sets of metabolites in body fluids and tissues that reflect normal and disease states, and to study interactions with the gut microbial flora and environment factors [70-72]. Special attention is being paid increasingly to lipidomics [73,74] and glycomics [75,76] as complementary sources of biomarkers.

The development of each of these global high-throughput technologies has triggered implementation of standard operating procedures, ontologies and quality-assurance pipelines for data collection and analysis using dedicated software and databases, and this has required a change in culture in biological laboratories [77-81]. In turn, the need for independent validation of the results obtained with these 'omics' technologies has stimulated the emergence of large-scale chemical-genetics and functional screens using cell microarrays and RNA interference [82-84].

Computational and mathematical tools empower systems biology

With the increasing availability of large amounts of data and curated information on all types of biological system components, the focus has progressively shifted to identifying the interactions they make, forming transient or permanent macromolecular structures with particular biological functions, and to looking at how the interactions can be represented computationally as metabolic, protein, microRNA and gene-regulatory networks [85,86]. This emerging 'network biology' is taking advantage of advances in functional genomics, computational methods, computing power, and network and graph theories. It is reviving the advanced biochemistry that has been published in textbooks and illustrated in static wall charts for decades [87-89]. Network biology is revealing the existence of modular structures in biological networks that may explain the robustness of biological systems when they are exposed to changing environments [90-93].

The initial attempts to identify biologically relevant protein-protein interactions using the yeast two-hybrid technology were plagued by high rates of artifactual events. Thanks to methodological improvements, the rate of false positives has been reduced. The careful curation of these interactions from targeted assays reported in the literature has led to high quality but incomplete maps of the human interactome, which are now available and which are expected to be extended to more complete coverage in the future [94-96]. Given that biological networks change their architectures dynamically during biological processes, such as development, physiological responses and disease, their complete determination will continue to be an enormous scientific and technological challenge.

Similar progress is being made in assembling human signaling, metabolic and gene regulatory networks that are based on metabolites, RNA and microRNA expression, protein-protein and protein-DNA interactions [97-100]. This has required the development of standardized languages and software tools for graphical representation of molecular interaction maps and computation of predictive and dynamic models [101-105]. Integration methodologies have also been essential to combine diverse types of data that have been collected with different platforms and in many laboratories, and thus to generate testable hypotheses [106-110]. A limitation that is often overlooked is that the quality of the annotation resources is very variable [111-114]. This has triggered sustained community efforts for integrative annotation, which combine automated computation with human-supervised curation, the use of

quality indices, text-mining tools, biological ontologies and the semantic web [115-120].

In general, the models derived from these integrated methodologies have not yet reached the level of detail and precision of those obtained through highly focused systems biology approaches, such as those that describe the transcriptional control in a free living microorganism under changing environmental conditions [121] or the early phases of development of the sea urchin [122]. It seems likely that the same operating principles of network structure and dynamics that have been revealed in these latter model systems will be relevant to human physiology and pathology [123].

In a parallel track, the Physiome Project is building on over half a century of molecular modeling of excitable cells that used ordinary and partial differential equations and is also using finite element lattices for geometric modeling of complete human organs. This project has steadily developed a computational physiology framework with its own modeling language [124,125], and initial models of the beating heart, the contracting muscle and the breathing lung are already available [126,127]. Cell and development simulation efforts use yet other types of modeling formalisms and languages, including Boolean networks, cellular automata and process algebra [128-135], and many others are being developed in computational neuroscience, which has yet to merge with systems biology [136].

This diversity of approaches for modeling biological systems highlights the renewed importance of the contributions of mathematics, informatics and physics to systems biology [137-139]. Despite the introduction of novel computational methods, given that they are often based on distinct or incompatible principles, it is difficult or impossible to integrate these methods across the multiple levels of organization and time-scales characteristic of living systems [140-142]. Thus, multi-scale integration of different types of biological information (DNA, RNA, protein, networks, organelles, cells, tissues, organs, higher level phenotypes, and so on) remains a major challenge in systems biology. The plea for more theory by some of the founders of systems biology must be tempered by the fundamental need to have theories that closely reflect biological data through hypothesis-driven model testing [143]. Recent proposals based on allometric scaling [144] and scale relativity theory [8,145] may provide the theoretical framework and mathematical tools required to overcome some of these limitations, and may reveal an important role for small fluctuations in driving the behavior of biological systems [146,147].

The transition from medical genomics to systems medicine

With the availability of increasingly powerful high-throughput technologies, computational tools and integrated knowledge bases, it has become possible to establish new links between genes, biological functions and a wide range of human diseases [148-153]. This is providing signatures of pathological biology [154] and links to clinical research [155] and drug discovery [156,157]. These are the hallmarks of systems medicine as it is emerging from the initial, more targeted efforts of medical genomics.

Success in the identification of mutations affecting the hundreds of genes involved in inherited disorders has been a major outcome of the first generations of genetic maps of the human genome. In contrast, the reported associations between genetic polymorphisms and common complex traits have rarely been confirmed in independent studies. The situation has changed in the past two years [158], with the availability of dense maps of single nucleotide polymorphisms and the adoption by the community of medical geneticists of consensus guidelines for the optimal design of genome-wide or targeted association studies, including rules for independent replication [159,160]. Despite the very significant problems with signal-to-noise ratios that still severely limit the conclusions that can be drawn from such studies, progress has been made in identifying susceptibility loci involved in, for example, diabetes [161-163], obesity [164], and breast or lung cancer [165-167]. In the case of lung cancer, however, different scientific groups interpret the functional significance of the results differently. Further progress is expected now that the important role of other forms of genomic polymorphisms between individuals, including monozygotic twins, has been recognized; these include the effects of copy number variations and epigenetic modifications [168-170].

Taking advantage of expression-profiling surveys performed in extended human populations [171-174], systems biologists have started integrating physiopathology, network biology and DNA variations [175-177], providing novel insights into the mechanisms of various diseases, such as diabetes [178] and obesity [179]. Cancer, which can be considered as a prototypical systems disease, has benefited greatly from systems approaches and has served to a large extent as a test case to develop them [180-184]. This work has highlighted the importance of epigenetic variations in controlling transcriptional programs sustaining differentiation of normal and cancer stem cells [185,186].

Transcriptome and proteome analyses of collections of cancer samples, combined with functional annotation and modeling of modulated molecular pathways and networks, have revealed useful biomarkers for the classification and diagnosis of cancer subtypes, the prognosis of patient outcomes, the prediction of treatment responses and the identification of perturbation targets for drug development [187-196]. As an illustration of the value of systems approaches, the predictive power and robustness of biomarkers can be significantly increased by integrating transcriptome profiles with

interactome data to reveal more relevant functional subnetwork modules [197]. In a similar way, systems approaches are starting to have an impact on the study of immunological diseases [198], inflammation [199], infectious diseases such as tuberculosis [200], neurological diseases such as autism [201] and Alzheimer's [202], respiratory diseases such as asthma [203], cardiovascular and metabolic diseases [204-206] and many others. A common biological theme that emerges from many of these studies is that the control and dysfunction of energy metabolism has a central role. This is illustrated in cardiac system bioenergetics by the Frank-Starling law of cardiac muscle contraction [207,208], in cancer by the Warburg effect (the dependence of cancer cells on aerobic glycolysis) [209], and in neurodegenerative diseases and aging by increases of oxidative stress [210,211].

When East and West, North and South meet to develop systems medicine

Systems approaches are likely to help elucidate the mechanisms underlying the fundamental biological processes perturbed in human diseases and, in doing so, enable more efficient therapeutic interventions. They will change how drug targets are identified. Novel treatments will include multiple drugs interacting with key interconnected components within functional network modules, each contributing a fraction of the effects of perturbations that cause disease. It is likely that they will be effective only when combined with the multiple interactions of other drugs. This reflects the way that biological systems function and are organized to maintain themselves and constantly adapt to developmental, environmental, physiological or pathological changes. It is also reminiscent of the principles underlying traditional medicines developed empirically within Chinese or Indian cultures for the past several thousand years. Initial attempts at systems approaches, using transcriptome and proteome analyses to study the synergistic effect of combining Western drugs with Chinese medicine components in the treatment of leukemia, are starting to bear fruit [192,212,213]. Similarly, metabolome studies are being used to analyze the composition of herbal medicines and explain their properties [214], and to establish how gut microorganisms modulate human metabolic phenotypes and respond to the health or disease state of their host [215].

Systems approaches are also providing evidence on the effects of stress, relaxation, nutrition and lifestyle on the course of health and diseases [216,217]. Systems studies need to pay greater attention to gender, age and time differences in diet, disease development and treatment administration and responses [218-221]. These factors can be monitored, for example, using non-invasive metabolomics surveys of urine [222,223], and they will increasingly also be monitored using molecular fingerprints of blood proteins that indicate relevant physiological or disease states. Other important contextual phenomena that also need to be taken into account in future studies include the effects of the mother's genetic makeup or feeding habits on the development of the fetus and the timing of its biological clock, which have been observed in animal models [224,225], and the central role of the major histocompatibility complex in the development and control of disease through immunity and inflammation [226-229].

Thus, systems biology will provide the foundation for a prospective medicine that will be predictive, personalized, preventive and participatory [230], and that takes into account the multiple components of the healthcare system, including disease outcomes as reported by the patients themselves, and public and private organizations involved in healthcare management. [231]. In addition to genomics and systems biology, the key components that will ensure the successful development of systems medicine are the modeling of physiopathology in a clinical-practice context [232], imaging [233], and bio-banking that complies with strictly enforced ethical regulations [234-236]. These intrinsically interdisciplinary endeavors will require dedicated centers and networks in which scientists of all disciplines can work together [237-239], with careful attention to clinical practice and education [240,241].

In order to implement this vision, academia and industry will have to work closely together in an open-access and open-source environment focusing on the initial, pre-competitive phase of the drug discovery process. This will enable the subsequent development of valuable intellectual property that will result in more effective diagnostic and therapeutic approaches. Such developments might seem very far from the priorities of the less developed countries, in which the majority of the population is excluded from basic medical care. These countries are facing major challenges to their ability to fight infectious diseases and malnutrition, a situation aggravated by the shortage of safe drinking water and economic poverty [242]. International initiatives are underway to tackle these challenges in global health, such as support for engagement of communities in research and formulation of a research and development treaty that will redefine the rules for clinical trials and management of intellectual property rights [243,244].

Strategic partnerships, such as the Systemoscope Consortium, propose guidelines and strategies for 'rethinking research, understanding life, improving health' [245]. We support the view that leaders of the developing countries should consider establishing integrative systems biology and medicine centers networked with those emerging in the developed countries. Implementing such centers at the heart of their much-needed healthcare infrastructures would ensure immediate access to the most advanced technologies, and allow developing countries to build an essential

knowledge base centered on the analyses of their populations. These centers would provide a route to the adequate healthcare that is required to reduce the ever-growing gap between the developed and underdeveloped nations.

Competing interests

CA is Research Director at CNRS, and a consultant for bioMérieux and Mérieux Alliance. ZC is the Director of the Shanghai Center for Systems Biomedicine and is supported by the National Key Program for Basic Research (973), China. LH is the President of the Institute for Systems Biology.

Acknowledgements

We thank our colleagues of the Genexpress team, the Center for Systems Biomedicine, the Institute for Systems Biology, and Haim Bendayan, Samir Brahmachari, Anthony Brookes, Dominique Charron, Eric Eveno, David Galas, Takashi Gojobori, Sandrine Imbeaud, Doron Lancet, Jacques Mallet, Xavier Leverve, Laurent Nottale, Denis Noble, Christophe Pison, Valdur Saks, Marc Vidal, John Weinstein for insightful discussions.

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Exhibit 23

Genome Medicine

Commentary

Gene patents and personalized medicine - what lies ahead?

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Abstract

Gene patents have generally not impeded biomedical research, but some problems that arise in genetic diagnostics can be attributed to exclusively licensed gene patents. Gene patents for therapeutics have often been litigated but have received surprisingly little public outcry. In stark contrast, genetic diagnostics have been highly controversial but rarely litigated: no case has gone to trial and there is little case law to guide policy. Most recently the Secretary's Advisory Committee for Genetics Health and Society (SACGHS) released a draft report examining how patenting and licensing affect access to clinical genetic testing in the US. The SACGHS reported that patents neither greatly hindered nor facilitated patient access to genetic testing; both the harms and the benefits of patents on genetic diagnostics have been exaggerated. Problems do occur when patents are exclusively licensed to a single provider and no alternative is available. Courts have been changing the thresholds for what can be patented, and how strongly patents can be enforced. Technologies for sequencing, genotyping and gene expression profiling promise to guide clinical decisions in managing common chronic diseases and infectious diseases, and will likely be an integral part of personalized medicine. Developing such genomic tests may require mapping a complex intellectual property landscape and cutting through thickets of patented DNA sequences and related methods. Our preliminary studies have found patent claims that, if strictly enforced, might block the use of multigene tests or full-genome sequence data. Yet new technologies promise to reduce the costs of complete genomic sequencing to prices that are comparable to current genetic tests for a single condition. Courts, companies, and policy makers seem unlikely to allow intellectual property to obstruct such technological advance, but prudent policy will depend on careful analysis and foresight. The SACGHS report signals that the US government is paying attention, and increases the odds that policy will foster socially beneficial uses of genetic testing while preserving intellectual property incentives and mitigating the problems that arise from legal monopolies.

Introduction

In April 2009, the US Patent and Trademark Office (PTO) granted its 50,000th DNA patent, making at least one claim about DNA or RNA molecules or their uses [1]. A gene patent, as the term is generally used, is a DNA patent that claims rights over nucleic acid sequences encoding proteins, or variants of those sequences. Jensen and Murray [2] identified 4,270 US patents granted by the end of 2004 that explicitly claimed DNA sequences for 4,382 out of 26,688 (approximately 20%) human genes catalogued in the RefSeq database. Another study found 16,000 DNA-sequence patents were granted worldwide, with the US PTO granting almost ten times more such patents than either the European or Japanese patent offices from 1980 to 2003 [3].

Gene patents have been of particular interest because of their relevance to biopharmaceuticals and genetic diagnostics. Gene patents underpin production of therapeutic biologic products such as insulin, growth hormone, erythropoietin and other growth factors, or of specific antibodies for biological therapy. Some therapeutic gene patents have been the subject of litigation [4] but there has been relatively little public controversy, perhaps because such patents act much like those on small-molecule drugs.

In contrast, gene patents have repeatedly sparked controversy in the context of genetic testing for Mendelian disorders and mutations predicting risk of common chronic diseases. Media coverage has been predominantly negative. Testing *BRCA1* and *2* gene variants to predict inherited risk of breast and ovarian cancer has been particularly controversial, with overwhelmingly negative press coverage, except in Utah, home to Myriad Genetics, the company with exclusive rights to perform diagnostic testing [5]. In May 2009, a consortium of plaintiffs supported by the American Civil Liberties Union filed a lawsuit in federal court in New York against Myriad Genetics and other defendants [6]. Because this lawsuit challenges the validity of claims on isolated nucleotide sequences, the final ruling on the suit could affect the validity of numerous other patents with similarly structured claims for genetic diagnostics as well as therapeutics. Concerns raised about the effects of gene patents on diagnostics include sole provider companies' ability to set a *de facto* national standard of clinical care, higher costs, lower quality, inhibition of better testing methods, impediments to clinical research, and limitations on medical professionals' education.

PTO, Patent and Trademark Office; SAGGHS, Secretary's Advisory Committee on Genetics Health and Society.

Studies and policy reports on gene patents

Over the past decade, the UK's Nuffield Council of Bioethics [7], the Australian Law Reform Commission [8], the Organization of Economic Co-operation and Development [9] and the European Society of Human Genetics [10] have prepared policy reports on how patents affect genetic diagnostics. Several case studies suggest that patents and exclusive licensing have reduced the availability of genetic testing for hereditary hemochromatosis, Canavan's disease, and breast cancer [11-17]. In addition, responses to laboratory surveys indicate that patent enforcement has reduced the number of providers for some patented genetic tests [11,17].

The Secretary's Advisory Committee on Genetics Health and Society (SACGHS, US Department of Health and Human Services) is preparing a policy report addressing the effects of patenting and licensing on clinical access to genetic testing in the United States. The SACGHS released a draft report for public comment in March [18], and is likely to release the final report late this year or early in 2010. Our group at Duke University gathered empirical evidence for the SACGHS at the committee's request. We concluded that it is difficult to make evidence-based claims that patents have either substantially helped or hindered clinical and patient access to genetic testing in the US [19].

It is not just what is patented that matters, but how patents are used

Patenting and licensing of patents relevant to DNA diagnostics interact in complex ways with the US healthcare system. Problems arise when patents covering genetic tests are licensed exclusively to a single test provider and no alternative avenue for testing is available. When the provider does not offer all forms of genetic testing (for example, preimplantation or prenatal testing or testing of paraffin-embedded tissues), or does not have coverage and reimbursement agreements with insurers or health plans, patients cannot turn elsewhere to get a test. Moreover, firms with *de facto* monopolies on testing for certain conditions have no explicit policies to clarify that they support basic and clinical research, verification of results and 'second opinion' testing, and transparent, independent proficiency testing and quality control. It bears emphasis, however, that case studies of current genetic testing cannot fully predict what issues are likely to arise for future DNA diagnostic technologies.

Genomic diagnostics: past experience may not predict future uses

Technologies for genotyping, gene expression profiling and full-genome sequencing are advancing with extraordinary rapidity, promising to displace genetic testing methods that have been fairly stable for a decade. New classes of diagnostics can simultaneously detect millions of genetic variations and mutations, and expression-level changes in thousands of genes, or even the entire genome. Genomic diagnostics are beginning to realize the promise of personalized medicine as they increasingly guide choices among treatments, drug doses and preventive interventions for cancer, heart disease, diabetes and infectious diseases.

Navigating the complex intellectual property landscape of DNA patents poses challenges for genomic diagnostics. Multi-gene diagnostic tests may infringe existing DNA-sequence or method claims, and difficulty in securing freedom to operate could slow some promising clinical technologies. Concerns include difficulties in aggregating rights to many patent claims and costs of procuring multiple licenses (royalty-stacking), often described as the anticommons effect, and securing rights already exclusively licensed to others by prior agreement (blocking problems). To date, less than 1% of US 'gene patents' have spawned litigation. This frequency is comparable to patents in general (1 to 2%). Only five cases have involved genetic diagnostics and all five were settled before going to trial [4]. The rarity of litigation and the complete absence of precedent-setting case law for DNA diagnostics, however, does not imply that patents have no effect. In many cases, it is clear that simply sending letters threatening patent enforcement is sufficient to 'clear the market' of competitors in genetic testing [20]. Litigation is very costly, and many of the relevant economic effects may be lower than the threshold cost of either bringing or fighting a lawsuit. As DNA diagnostics become more valuable and the stakes get big enough to fight about, litigation might become more common. Moreover, the few cases of litigation have involved traditional genetic testing, and not the emerging multi-gene diagnostics. Those developing such new diagnostic technologies are flying in the dark.

Shifting jurisprudence

Uncertainty about DNA diagnostic patents is compounded by shifts in patent practice and jurisprudence. The US PTO announced higher thresholds for utility and written description of sequence-based patent claims in 1999 and implemented them in early 2001 [21,22]. Recent decisions by the US Court of Appeals for the Federal Circuit and the US Supreme Court have raised the standard for obviousness (*KSR International Co.* v. *Teleflex Inc.*) [23], changed criteria for allocating damages for patent infringement proportional to the actual contribution of the patented invention under dispute, and made automatic injunctions more discretionary (*eBay Inc.* v. *MercExchange LLC*) [24]. Two pending cases, *Bilski* v. *Kappos* (expected to be decided by the Supreme Court in 2010) [25], and the recent Appeals Court decision in *Prometheus* v. *Mayo* [26] promise to be directly relevant to DNA diagnostics, and could change the game. In Europe, there has been even less litigation over diagnostics, and far less patent enforcement than in the US, but commercial genetic testing may become more important there in coming years [27,28].

The future of patents and diagnostics: continued uncertainty and increasing complexity

Our preliminary analysis suggests that microarray-based methods of genetic analysis for many genes and gene variants, and *a fortiori* full-genome sequencing could arguably infringe on some patent claims. However, such claims have never been tested in court, and some appear vulnerable to changing interpretations of what can be patented, so it is difficult to know if they would be held valid if challenged. While much of the attention has focused on patents claiming nucleic acid sequences, claims on specific methods for analyzing or detecting DNA variants are at least as important. In a recent analysis of claims for patents associated with 22 commonly administered genetic tests, van Overwalle and colleagues [29] determined that method claims covering these diagnostics could be harder to 'invent around' than claims on cDNA sequences. A 2006 study [30] showed growth in patents claiming research tools such as methods for gene expression profiling or detecting single nucleotide polymorphisms and even algorithms and software for disease prediction, classification and prognosis. Some patents claim associations between DNA sequences and clinical conditions or medical outcomes and could also prove difficult to work around.

There is no consensus about whether DNA sequence patents hinder or help in the development and availability of genetic diagnostics, and empirical evidence about how these patents may affect commercialization of new genomic diagnostics is scarce. This seemingly innocuous statement is an advance in the public debate, given that the alleged negative consequences and benefits of patents have so often been grossly overstated. The framework for genetic testing is likely to change within the next decade, as the cost of individual whole-genome sequencing drops to levels comparable to current genetic tests for individual conditions. The forthcoming SACGHS report may have a salutary effect on norms and practices in patenting and licensing of technologies relevant to emerging DNA diagnostics, merely by shining a spotlight on them.

Competing interests

The authors declare that they have no competing financial interests. The authors were part of a team of researchers that gathered empirical evidence for the SACGHS draft report.

Authors' contributions

SC and RCD both contributed to the design and writing of this commentary.

Acknowledgements

The authors gratefully acknowledge the support of the National Human Genome Research Institute and the Department of Energy (CEER Grant P50 HG003391), Duke University, Center of Excellence for ELSI Research. The authors also thank Arti K Rai for helpful comments and criticism. The authors also acknowledge Christopher Heaney for useful comments and help with editing.

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Published: 28 September 2009
doi:10.1186/gm92
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Exhibit 24

Legal uncertainty in the area of genetic diagnostic testing

Isabelle Huys, Nele Berthels, Gert Matthijs & Geertrui Van Overwalle

A patent landscape analysis of 22 common genetic diagnostic tests shows substantially fewer claims on genes *per se* than initially suggested but raises questions of legal uncertainty as to the claims' scope.

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Ever since genes were first patented, the legitimacy and economics of human gene patents have been at the forefront of intense debate[1]. Different stakeholders have expressed concerns about the effect of 'blocking patents' or 'patent thickets' on genetic technology, arguing that because such claims are difficult or impossible to circumvent, they would increase genetic testing prices and hinder innovation[2,3]. The debate has been directed towards the creation of possible solutions for the potential "tragedy of the anticommons" and several collaborative licensing models have been proposed[4].

At present, a few studies provide empirical information on the granting[5–8] or litigation of gene patents[9]. Some of these studies primarily analyze anecdotal cases[10,11], whereas others examine the impact of gene patents more widely[12–15]. Some further studies suggest that the patent thicket may emerge more manifestly in the diagnostic sector, resulting in an undersupply of diagnostic testing services or the development of suboptimal diagnostic tools[16].

What has been lacking is a large-scale empirical study that defines the heart of the problem: which types of claims occur in disease-specific patents and to what extent are these claims essential for carrying out genetic diagnostic tests?

The present study aims to unravel on a qualitative as well as quantitative basis what is claimed in US and European patents on the 22 inherited diseases most frequently tested for in Europe (Table 1). This research provides an in-depth analysis of patents, investigating the exact number, status, nature and scope of granted disease-specific patents that are in force. The results from this study will be valuable for future research regarding licensing models that facilitate access to blocking patents and patent thickets because, depending on the nature of the claimed subject matter, the appropriate licensing models differ.

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Methodology

Scientific focus. This work constitutes an analysis of the nature, extent and scope of patents related to genetic diagnostic testing of 22 inherited diseases. The choice of these diseases results from a literature study in addition to consultations with practitioners in the field of genetic testing and the diagnostics of rare diseases, leading to a systematic ranking of the diseases according to their prevalence and clinical importance in Europe (Table 1). Only monogenic, disease-specific patents were included in the study, meaning that patents on overall detection methods were disregarded, as well as multifactoral genetic diagnostic testing activities and diagnostic testing using protein analysis.

The problem of access affects the genetic diagnostic testing providers, who may be faced with specific restrictive licensing practices hindering genetic testing services based on the patents they work on, as well as the patient, who may, for example, be confronted with increased prices for services. This research considered the access of genetic diagnostic testing services to genetic diagnostic patents. The present paper does not relate to the effects of patents on future research initiatives or the development of specific kits. Neither does the study assess whether there is an underuse or even no use at all of patented genetic technology by diverse testing providers[14].

Patent search. Patent applications and granted patents relating to these 22 diseases were extensively searched in both public and commercial patent databases, using the algorithm largely as described earlier[15]. Our strategy comprised searches based on the international patent classification (IPC) codes or European classification (ECLA) codes and searches based on sets of keywords best describing the genetic technology at stake. Additional literature reviews and relevant case law completed the picture. The patent information provided here was last updated on February 6, 2009. Of course, this study does not intend to replace the freedom-to-operate analysis that users would have to do.

Legal status. The legal status of the European and Patent Cooperation Treaty patent documents was checked on the European Patent Office's (EPO's) site Epoline (http://www.epoline.org) and EspaceNet (http://www.espacenet.com/index.en.htm). The legal status of US patent documents was found on the United States Patent and Trademark Office (USPTO)'s patent application information retrieval website (http://portal.uspto.gov/external/portal/pair) and the main USPTO website (http://www.uspto.gov). For the aim of this study, four legal-status categories were used, namely, (i) patent applications pending, (ii) patents granted, (iii) patent applications dropped and (iv) 'dead' patents, which means patents that were abandoned, withdrawn or deemed to be withdrawn, revoked or out of term. With regard to the patent

Table 1 Disease characteristics and best-practice guidelines for genetic diagnostic testing

Genetic disease	Abbreviation; OMIM[a]	Gene	Locus	Best practice guidelines
Fragile X	FRAXA; OMIM 300624	*FMR1*	Xq27.3	Footnote 1
Cystic fibrosis	CF; OMIM 219700	*CFTR*	7q31.2	Ref. 35
Familial breast and ovary cancer	BRCA; OMIM 114480	*BRCA1, BRCA2*	17q21, 13q12.3	Footnote 1
Hereditary hemochromatosis	HFE; OMIM 235200	*HFE*	6p21.3	Footnote 1
Factor V Leiden thrombophilia	FVL; OMIM 188055	*F5*	1q23	Ref. 36
Factor II thrombophilia	FII; OMIM 176930	*F2*	11p11-q12	Ref. 36
Beta thalassemia	HBB; OMIM 141900	*HBB*	11p15.5	Footnote 1
Hereditary nonpolyposis colon cancer	HNPCC; OMIM 120435	*MSH2, MLH1, MSH6, PMS2*	2p22-p21, 3p21.3, 2p16, 7p22	Footnote 1
Prader-Willi syndrome	PWS; OMIM 176270	*UBE3A, SNRPN, NDN*	15q11-q13	Footnote 1
Angelman syndrome	AS; OMIM 105830	*UBE3A*	15q11-q13	Footnote 1
Charcot-Marie-Tooth neuropathy 1A	CMT1A; OMIM 118220	*PMP22*	17p11.2	Footnote 1
Myotonic dystrophy	MD; OMIM 160900	*DMPK*	19q13.2-q13.3	Footnote 1
GJB2-related hereditary hearing loss and deafness	KID; OMIM 148210	*GJB2*	13q11-q12	Footnote 1
Duchenne muscular dystrophy	DMD; OMIM 310200	*DMD*	Xp21.2	Footnote 1
Huntington's disease	HD; OMIM 143100	*IT15*	4p16.3	Footnote 1
Spinocerebellar ataxia	SCA; OMIM	*ATXN genes*	Multiple loci	Footnote 1
Spinal muscular atrophy	SMA; OMIM 253300	*SMN1, SMN2*	5q12.2-q13.3	Footnote 1
Familial adenomatous polyposis	FAP; OMIM 175100	*APC*	5q21-q22	Footnote 1
Attenuated familial adenomatous polyposis	AFAP; OMIM 175100	*APC*	5q21-q22	Footnote 1
Facioscapulohumeral muscular dystrophy	FSHD; OMIM 158900	*FRG1, FRG2*	4q35	Footnote 1
Friedreich ataxia	FRDA; OMIM 229300	*FXN*	9q13	Footnote 1
Rett syndrome	RTT; OMIM 312750	*MECP2*	Xq28	Footnote 1

The diseases are ranked in descending order with the most commonly tested disease in Europe on top.
[a]Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim/).
Footnote 1: http://www.eurogentest.org

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applicants, original ownership positions were investigated, and each individual applicant has been counted once to get a real view on different types of applicants. To address the relevant territorial scope, we have included information on the country of destination for the European patents and patent applications. Admittedly, the figures in this study represent a one-moment shot of the patent landscape in genetic diagnostic testing. As the legal status of the patents examined will change over time, the landscape may be altered within a few months.

Claim analysis–typology. Only the active and granted patents within the patent landscape were further analyzed and categorized with regard to the scope of the protection they claim, namely, whether the patents claim (i) polynucleotide sequences ('gene' category), (ii) primers or probes ('oligo' category), (iii) genetic diagnostic methods ('methods' category) and (iv) genetic diagnostic kits ('kit' category), as most concerns with regard to patent thickets originate from these categories. Our attention has been directed to the independent claims because these define, technically, the broadest scope of the patent protection.

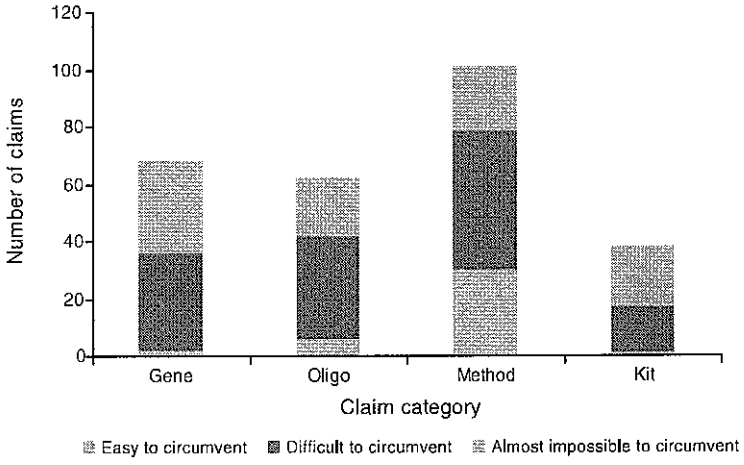

Figure 1 Claim typology and impact. One hundred forty-five active granted patent claims were analyzed and categorized in four categories (gene, oligo, methods and kit category).

Claim analysis–impact. The claims were analyzed for each and every one of the granted, active European and US patents. For European patents, Art. 83, Art. 69 and the Protocol of the European Patent Convention (EPC) were used as the basis for an interpretation, which, according to the EPC's exact wording, combines a "fair protection for the patentee with a reasonable degree of certainty for third parties." For US patents, the basis for claim interpretation was found in the US Utility Patent Act §112, asking for a "clear written description" and "best mode for carrying out the invention," as well as specific case law[17]. The claims at hand were interpreted in the light of the

patent specification, the drawings and the other claims and were compared to the best practices guidelines (when available) for the testing of susceptibility to these genetic diseases in Europe and the United States, to assess the essentiality (i.e., the neccessity of having access to the technology) of the specific patent claims for carrying out a genetic diagnostic test using those guidelines. By applying this methodology, three impact levels were distinguished, and a color was attributed to indicate whether the currently practiced genetic diagnostic tests are covered to a minor degree (green), partly (orange) or completely (red) by the claims (Fig. 1). 'Green claims' can easily be circumvented by using a different genetic diagnostic testing technique. 'Orange claims' can be circumvented but this requires a substantial investment of money and time, as well as a large amount of inventiveness. 'Red claims' are almost impossible to circumvent and are therefore also called 'blocking claims'.

It should be noted that two additional aspects have not been scrutinized, namely an investigation of the file history—that is, the complete file of a patent application containing all related papers prepared by the patent office and the applicant during the patent prosecution—and a comparison of a specific actual diagnostic method with the claims. Therefore, in the context of the present contribution, no full freedom-to-operate analysis has been carried out.

Licensing policy. By way of example, for some specific genetic disease patents, the licensing policy has been investigated by directly contacting the respective licensor or licensee. A more extensive analysis of the licensing policy goes beyond the scope of this study.

RESULTS

Patent search and legal status

Number of patent documents (Europe, United States). The disease-specific patent searches yielded more than 250 relevant patent documents in 72 different patent families. A patent family is a group of patents taken in various countries to protect a single invention (Fig. 2).

1. Granted patents. US patent documents appear in 66 patent families, whereas European patent documents were present in only 26 patent families (Fig. 1). European and US patent documents occur together in 21 patent families, whereas 46 patent families contain only US patent documents. Five patent families have European patent documents but lack a US counterpart.

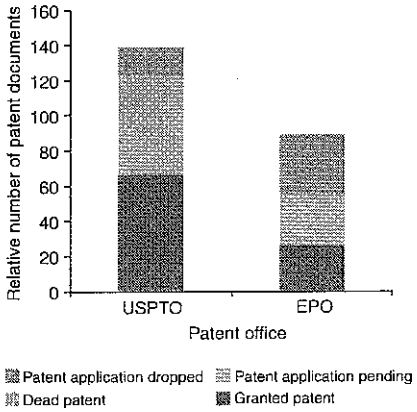

Figure 2 Legal status and relative number of patent documents within the genetic diagnostic patent landscape. The figure shows only one patent document per patent family, and one patent family may figure in different categories. "Patent application dropped" means that the application is no longer under examination (abandoned, refused, withdrawn or deemed to be withdrawn) in the national (US) or regional (EP) phase. "Dead patent" means that a granted patent has either lapsed or been nullified. "Patent application pending" is a patent application currently under examination in the national (US) or regional (EP) phase. "Patent granted" is a granted patent, currently active and valid.

2. Patent applications. Patent applications are distributed similarly, as 42 patent families are filed with the USPTO, whereas about half of this number, 22 patent families, were filed with the EPO.

3. 'Dead' patents. In the United States, 15 patent families are quoted as no longer in force, whereas in Europe, this number is only 7. However, relative to the number of granted patents, figures on patents no longer in force are almost similar in Europe (27%) and in the United States (22%).

4. Patent applications dropped. Remarkably, about twice as many patent applications are dropped during the patent prosecution procedure in Europe (34) compared to the United States (16).

Among the patent documents handled at the EPO, granted patents (26) and pending patent applications (22) are about equal in number. In the United States, however, the relative number of granted patents (66) is about 1.5 times the number of pending US patent applications (42).

Patented genetic diagnostic tests. Figure 3 shows that 19 out of the 22 genetic diseases under study are covered by patents or patent applications in Europe and/or in the United States. In Europe, four diseases are covered by granted patents only, whereas in the United States, six diseases are. Most heavily patented in Europe are hereditary hemochromatosis and familial breast cancer testing. In the United States, most patents are for spinocerebellar ataxia, Charcot-Marie-Tooth neuropathy, hereditary nonpolyposis colon cancer and familial breast and ovary cancer.

In Europe, four diseases are covered by pending patent applications only, whereas in the United States, only facioscapulohumeral

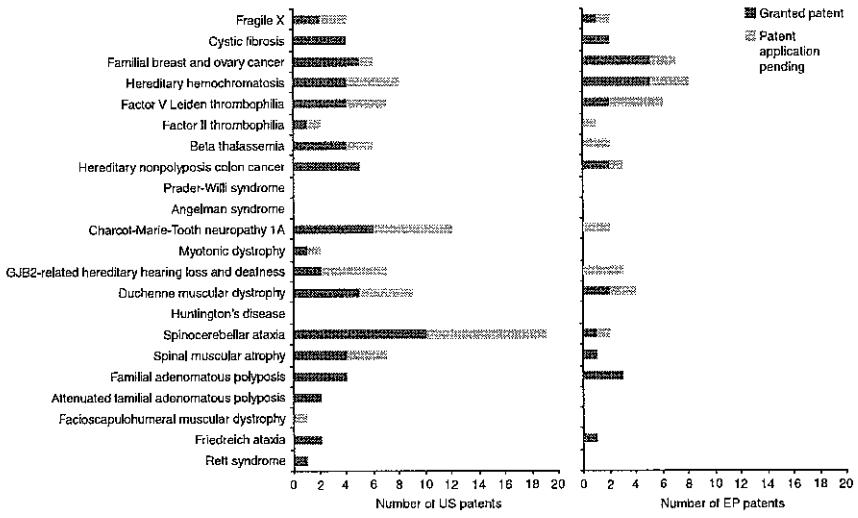

Figure 3 Legal status and relative number of patent documents for the top 22 diseases. Granted patent is a currently active and valid patent. Patent application pending is one currently being examined.

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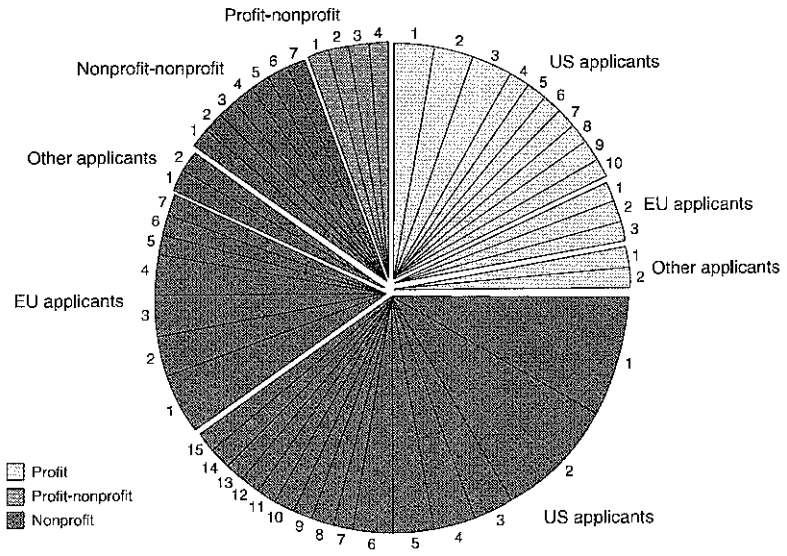

Figure 4 Patent applicants according to their nationality, type of ownership (profit or nonprofit) and number of patent families (as shown by the area of the slices). "Nonprofit" means universities, research institutes, universities and colleges, governments and private persons; "profit" means companies; "nonprofit-nonprofit" means co-ownership by two or more nonprofit organizations; "nonprofit-profit" means co-ownership by two or more nonprofit and profit organizations. Other applicants: as reflected in **Supplementary Table 1** (IN, Ca, JP).

muscular dystrophy (FSHD) is covered by patent applications only.

In Europe, genetic diagnostic testing for 7 out of the 22 hereditary diseases (31.8%) is covered both by patents and patent applications, whereas in the United States, genetic diagnostic testing for 12 out of the 22 hereditary diseases (54.5%) is covered by both granted patents and pending patent applications.

Profile of patent applicants. In total, 56 different applicants were registered (Fig. 4). Most applicants (62.5%) originate from the nonprofit sector. The profit sector represents the remaining 37.5%.

Across the sectors, the predominance of US applicants is striking: 58.9% of applicants originate from the United States, only 25% of applicants come from Europe. The remaining 16.1% comes from Japan or Canada. The US applicants own 55.8% of the identified European patents, whereas 20.5% of European patents is owned by European applicants and the rest belong to Canadian or Japanese applicants. US applicants hold 67.0% of the identified US patents, whereas 8.5% of these are held by European applicants and the rest by Canadian or Japanese applicants.

The nonprofit sector takes the lead with 60% different, (i.e., we considered each applicant once in the calculation of the percentage) US applicants, versus 28.5% different European applicants and 11.5% from other countries (Japan, Canada). In the profit sector, 57.2% different applicants originate from the United States, compared to 19.0% different European applicants and 23.8% from other countries.

The study revealed 11 different co-ownerships representing 17 different partners. In total, 30.3% different applicants have patents in co-ownership. Seven co-ownerships consist solely of nonprofit partners, and four were mixed co-ownerships (from profit and nonprofit sectors). Within the profit sector, 28.5% of applicants hold patents in co-ownership (two US applicants, one Japanese applicant, two Canadian applicants and one UK applicant). In the nonprofit sector, 30.5% different applicants have patents in co-ownership (six from the United States, one from Canada, one from Japan, one from the United Kingdom and two from France). All except one co-ownership have at least one US applicant, usually from the nonprofit sector: 41.1% different nonprofit US co-applicants compared to 11.7% from the profit sector. Almost half of the co-ownerships contain a European applicant, representing 17.6% different nonprofit applicants and 5.8% different profit applicants.

Top applicants in the United States are Johns Hopkins University (Baltimore) and Baylor College of Medicine (Houston; six patent families each). The top applicant in Europe is Leiden University (The Netherlands) with three patent families.

Assignments. Fourteen patent families originally filed by US nonprofit applicants were assigned to another US partner, whereas seven US profit organizations assigned their patent to another US partner.

Licensing. In the United States, most genetic diagnostic patents are exclusively licensed out to Athena Diagnostics (Worcester, Massachusetts, USA). This is the case for Fragile X, myotonic dystrophy, SCA, Duchenne myotonic dystrophy, FSHD, Charcot-Marie-Tooth neuropathy, GJB2-related hereditary hearing loss and deafness, and Rett Syndrome. Baylor College of Medicine granted some nonexclusive licenses for its patents. In Europe, the situation is far from clear[18].

Claim analysis

Claim analysis—typology. Out of the 250 investigated patent documents, 145 granted active patents (118 US patent documents and 27 European patent documents) were identified. Among the 145 patent documents, 267 independent claims were meticulously analyzed and classified into four categories (Fig. 4). In Europe as well as in the United States, most claims were directed towards methods (38%), followed by gene (25%), oligo (23%) and kits (14%). Most gene claims were directed towards 'isolated' cDNA (included in 66 out of 145 patents). Claims on genomic DNA, however, appeared in 22 patents.

Claim analysis—impact. The 267 independent patent claims were further classified according to their impact on genetic diagnostic testing (Fig. 1). Nearly half of the claims can be regarded as difficult to circumvent (orange). About a third of the claims (36%) can be regarded as easy to circumvent (green). A share of 15% was considered as almost impossible to circumvent and thus 'blocking' (red), that is, 35 out of 145 patents contain at least one blocking claim (Table 2), mostly a blocking methods claim (Fig. 1).

Within the gene claim category, only 3% of the claims are considered blocking, and the majority of claims are easy or difficult to circumvent. Several patents on genetic sequences do not explicitly disclose the exact nature of the sequence in the claim, as demonstrated by claim 1 of US 6984487 (**Supplementary Discussion**) on the cystic fibrosis gene (**Supplementary Information**). Depending on the specific situation, these claims were classified as difficult or impossible to circumvent.

Within the methods claim category, the impact is less extremely distributed, in that

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claims that are almost impossible to circumvent, claims that are difficult to circumvent and claims that are easy to circumvent are present in 30%, 47% and 23% of the patents, respectively. In many instances the exact method of genetic diagnosis is not specified by the claim language and broad wordings are used, exemplified by the first claims from US 5693470 (HNPCC) and EP1015628 (SCA6) (Supplementary Discussion). Depending on the specific situation, these claims were classified as difficult or impossible to circumvent.

Oppositions and litigation suits

Several proceedings have been brought before the boards of EPO, relating to patents on familial breast and ovary cancer[19] (EP 699754 (T80/05), EP 705902 (T1213/05), EP 858467 (T902/07) and EP 705903 (T666/08) (cases as indicated with T numbers[19]). These patents have been drastically reduced in scope[20]. Two patents related to Factor V Leiden thrombophilia have also been the subject of an opposition procedure. Patent

Table 2 Claim analysis of patents

Genetic disease[a]	Patent number	Country of designation	Current assignee	Filing year	Type of claims			
					Gene	Oligo	Method	Kit
FRAXA	EP 580621	DE, ES, GB, IT	Inserm (FR)	1992	Y	Y	Y	Y
CF	US 6984487		HSC (CA)	1993	Y	Y	Y	Y
	US 5407796		NIH (US)	1991	N	Y	Y	N
	US 5693473		Univ. Utah (US)	1995	Y	Y	N	N
	US 5747282		NIH (US)	1995	Y	Y	N	N
	US 5753441		Univ. Utah (US)	1995	N	N	Y	N
BRCA	US 6033857		Univ. Utah (US)	1998	N	N	Y	N
	EP 699754	DE, ES, FR, GB, IT, NL	Myriad Genetics (US)	1995	N	N	Y	N
	EP705902	DE, ES, FR, GB, IT, NL	Myriad Genetics (US)	1995	N	Y	Y	N
	EP 705903	DE, ES, FR, GB, IT, NL	Myriad Genetics (US)	1995	Y	Y	Y	N
	US 6955875		Billups R. (US)	2001	N	N	Y	N
HFE	US 6355425		Billups R. (US)	1999	Y	N	Y	Y
	US 6849399		Bio Rad (US)	1997	N	N	Y	N
	US 6518016		Univ. Leiden (NL)	1995	N	Y	Y	Y
	US 6043035		Univ. Leiden (NL)	1997	Y	N	Y	Y
FVL	EP 690991	AT, BE, CH, DE, DK, ES, FR, GB, IE, IT, LI, NL, SE	TAC (SE)	1994	N	N	Y	N
	EP 696325	AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE	Univ. Leiden (NL)	1995	N	Y	Y	Y
FII	US 5693470		NIH (US)	1995	N	N	Y	N
	US 5492808		NIH (US)	1993	N	N	Y	Y
HNPCC	US 6165713		Dana Farber (US)	1997	N	Y	Y	Y
	EP 760867	AT, CH, DE, ES, FR, GB, IT, LI, NL, PT	Dana Farber (US)	1994	Y	Y	Y	N
CMT1A	US 5306616		NIH (US)	1991	Y	Y	Y	Y
MD	US 5955265		MIT (US); UWales (GB)	1995	N	N	Y	Y
	US 6485908		Inst. Pasteur (FR)	2000	Y	Y	Y	Y
GJB2 (KID)	US 5998147		Inst. Pasteur (FR)	1998	Y	Y	Y	Y
SCA1	US 5834183		Univ. Minnesota (US)	1994	Y	Y	Y	N
	US 5741645		Univ. Minnesota (US)	1995	Y	Y	Y	N
SCA6	US 6303307		RDF (US)	1999	N	N	Y	N
	EP 1015628	BE, CH, DE, FR, GB, LI	RDF (US)	1998	N	N	Y	N
SMA	EP 711833	BE, CH, DE, FR, GB, IE, LI, LU, SE	Inserm (FR)	1995	Y	Y	Y	Y
	US RE 36713 E		Zeneca (GB)	1996	Y	Y	N	N
FAP	EP 569527	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE	ICI (GB)	1992	N	Y	Y	Y
FRDA	US 6150091		Inserm (FR), BCM (US)	1997	Y	Y	Y	N
	EP 885309	BE, CH, DE, FR, GB, IE, LI, LU	Inserm (FR)	1997	Y	Y	Y	N
RTT	US 6709817		H. Hughes Medical Inst. (US)	2000	N	N	Y	N

This table displays only the diseases for which patents containing claims that are almost impossible to circumvent were identified. Out of the 22 inherited diseases studied, 15 diseases fall into this category. Of the seven other diseases, three were not covered by patents at all, whereas four diseases were covered by patents containing claims that were easy or difficult to circumvent ('pink' or 'blue' color respectively). The colors indicate the essentiality of patent claims for performing genetic diagnostic testing according to the Best Practice Guidelines. Pink, easy to circumvent; blue, difficult to circumvent; green, almost impossible to circumvent. HSC, Hospital for Sick Children; NIH, National Institutes of Health; TAC, Thrombosis and Coagulation Aktiebolag; MIT, Massachusetts Institute of Technology; RDF, Research Developement Foundation; ICI, Imperial Chemical Industries; BCM, Baylor College of Medicine; Y, yes, claim present; N, no such claim present. AT, Austria; BE, Belgium; CA, Canada; CH, Switzerland; DE, Germany; DK, Denmark; ES, Spain; FR, France; GB, United Kingdom; GR, Greece; IE, Ireland; IT, Italy; LI, Liechtenstein; LU, Luxembourg; MC, Monaco; NL, Netherlands; PT, Portugal; SE, Sweden; US, United States of America.

[a]Abbreviations defined in Table 1.

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EP 690991 was amended, whereas EP 807691 remained unamended[21]. In the United States, litigation was started with regard to familial breast and ovary cancer[22,23]. Both cases were settled before substantive issues regarding patent validity or infringement were addressed[24]. In our study, claims were analyzed as amended.

DISCUSSION

This empirical study leads to several surprising observations. A first observation concerns the number of patents within the patent landscape. Most genetic diagnostic patents originate from the United States. This supremacy of US patents on genetic inventions finds its roots in the first US case law on biomedical inventions, where the US Supreme Court stated that "anything under the sun that is made by man" is patentable[25]. Since 2001 however, the USPTO raised the threshold on patentability of genetic inventions, requiring a "specific, substantial and credible utility"[26]. Our study shows that nowadays, patents are more easily granted for genes for which biochemical, biological or genetic data describing the function are included (of which we found 63). However, many key patents (11 in our study) still are directed to gene sequence as such, without substantial evidence as to what the gene does. With regard to methods claims, the criteria developed in *In re Bilski* (2008) further raise the bar for patent eligibility, and might influence granting practices on genetic inventions[27]. A flow chart for putting claims to the *Bilski* test has been assembled[28]. A claim on the use of a computer or other machine as a substantial step in a genetic test may reduce the risk of losing a patent grant.

In Europe, a low number of granted patents are recorded compared to the United States, and twice as many European patent applications are dropped during the application phase. This might arguably be a consequence of Europe's restrictive patent prosecution system and the reputation of being strict, lengthy and costly[29].

A second observation deals with the type of applicants. The study reveals that three-fifths of the identified applicants originate from the nonprofit sector. This indicates that universities as well as research institutes represent important sources of inventions in genetic technology. The predominance of US applicants among these reflects the progressive attitude on granting patents on biomedical inventions in the United States.

The third observation relates to the claim analysis. Three specific questions were answered. First, is a patent thicket, defined as many blocking patents from many different owners, emerging in the genetic diagnostic sector? With respect to genes, 25% of the identified patents, filed by different applicants, claim a human gene, suggesting the possibility of the existence of a patent thicket. In contrast to this relatively high number of gene claims, only 3% of these gene claims can be classified as occupying a blocking position. Therefore, a hindering patent thicket cannot be demonstrated.

Surprisingly, most claims were directed to genetic diagnostic methods. The larger part of these methods claims is classified as difficult or impossible to circumvent, and in addition, patents containing these methods claims are owned by different applicants. For these reasons, we can identify a patent thicket in this category.

Second, does the broad formulation of gene and methods claims cause problems? Regarding genes, the few blocking gene patent claims identified are formulated broadly in covering many types of genes. For this reason, until the patents have been disputed in court, the exact scope of the claims remains unclear, which causes legal uncertainty about the scope of the patents and whether or not the diagnostic service falls under the scope of a specific patent.

Regarding methods, similarly as with gene claims, broad formulations of these methods claims were observed. Diagnostic methods claims generally confer protection for a series of working steps. However, if the claim broadly formulates the link between mutation and disease, without specifying steps as to how this link is determined, it is unclear when a diagnostic testing method is infringing. This may be illustrated by claim 1 of US 5693470 (HNPCC) and EP1015628 (SCA6) (**Supplementary Discussion**), which refers to the use of "any" test. Although with regard to the patentability criteria it should be almost impossible to construe a claim so broadly that it would cover an indefinite number of tests, the examples show that nevertheless, such claims are granted.

Finally, does the unclear terminology influence gene and methods claims? Specific patent rules such as in the UPA §112 in the United States and Rule 27(1)(f) EPC and Rule 23e(3) EPC in Europe, prescribe an obligation to give sufficient description of the invention.

With regard to gene claims, most claims are directed to cDNA sequences. Although the US Court of Appeals for the Federal Circuit as well as the EPO Board of Appeal held that "a gene is a chemical compound, albeit a complex one"[30,31], these decisions do not guide professionals as to how unclear gene claims have to be interpreted. Nucleic acid sequences may appear as DNA sequences, cDNA sequences, RNA sequences, short fragments, primers, probes and so forth. The best practices guidelines for genetic diagnostic testing do not generally prescribe cDNA as a starting material for performing genetic diagnostic testing. Most protocols start from blood samples for the isolation of genomic DNA. To determine whether or not there is infringement of a cDNA claim by performing the genetic diagnostic test, it should be identified what acts fall under the term 'product' and under the prohibited term 'use' (Art. 27 of the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS)), which, in this area, is not straightforward. This may be exemplified by claim 1 of US 6984487 (**Supplementary Discussion**) on the cystic fibrosis gene towards a "purified DNA molecule, comprising a CFTR DNA sequence selected from a group of different DNA types." According to the European Biotechnology Directive 98/44/EC (ref. 30) (Art. 5(2)) and the US Utility examination guidelines, sequences are patentable if 'isolated' from the human body. The term 'isolated' was seldom found to be clarified in the description, and if a definition was provided, instead of providing clarity, the intended meaning was further obscured due to the use of unclear or broad terms in the definition as well, as exemplified in US5693473 (BRCA1), where for instance the unclear term "substantially" again appears in the definition and where it remains unclear what is meant by "its natural environment."

Also with regard to oligo claims, the problem of unclear terminology arises, as exemplified by claim 16 of US5622829 (BRCA1) on a pair of single-stranded DNA primers for determination of a nucleotide sequence of a BRCA1 gene. Making use of a primer thus seems legitimate, but it is not clear whether using genomic DNA present in a blood sample as a template during a genetic diagnostic test is covered by such a claim to a specific cDNA.

With regard to methods claims, Art. 53(c) EPC2000 codifies that methods performed on the human body are unpatentable[33]. Though most diagnostic methods are performed *in vitro* and thus patentable, the effect of this provision remains unclear in practice. In addition, some tests claim a "method of identifying a mutant gene." The question then arises whether such a claim is infringed when a mutant cannot be identified in the sample and to what extent the outcome of a diagnostic procedure is thus determinative for the infringement analysis.

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In conclusion, the present analysis and accompanying observations do not point to the existence of a wide patent thicket in genetic diagnostic testing. Rather, they highlight a problem of lack of transparency and clarity, leading to legal uncertainty. Neither case law nor patent legislation resolves the legal uncertainty related to patents on genetic inventions. In 2006, the US Supreme Court dismissed a judicial review on the case *Laboratory Corporation of America Holdings v. Metabolite Laboratories Inc.*[34], related to a patent claiming a "relationship between homocysteine and vitamin deficiency." As a substantial number of patent claims would have been affected by a decision in this case, this refusal further increased legal uncertainty, including in the genetic field.

The consequence of this high level of legal uncertainty is that either enormous risks are taken if genetic tests are performed without knowingly infringing a specific patent, or much time and energy goes into establishing patent landscapes and freedom-to-operate analyses or to efforts to use different techniques and methods that may eventually be below the state of the art that is clinically requested.

As this study shows that not that many blocking gene patents exist, proposals aiming at banning patents on human genes do not provide a plausible solution[24], unless the ban would be on patents for broad genetic diagnostic methods as such. For instance, the European Society of Human Genetics (Vienna) has recently recommended avoiding patenting of the pure link between a mutation and disease[2]. More attention should be paid to the licensing practices in a 'responsible' way[16,35,36]. Otherwise, the risk exists that the control by owners of patents containing those broad claims with respect to genetic diagnostic testing may in the future dissociate actual genetic diagnostic testing from genetic counseling and clinical investigation, which is to the detriment of progress of the genetic diagnostic service and public healthcare system.

Note: Supplementary information is available on the Nature Biotechnology website.

DISCLAIMER
The content of this paper is informational only and should not be substituted for legal advice.

ACKNOWLEDGMENTS
This research was supported by grant number G.0120.04 of the Fund for Scientific Research (FWO, Belgium) and EuroGentest, an EU-FP6 supported Network of Excellence contract number 512148 and the Vancraesbeeck Fund (K.U.Leuven, Belgium). Special thanks go L.-A. Johnson, I. De Baere, E. van Zimmeren and B. Verbeure for interesting discussions and helpful comments on the manuscript.

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