AMELAF

ASOCIACION MEXICANA DE LABORATORIOS FARMACEUTICOS

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TITULAR
COMISION FEDERAL DE MEJORA REGULATORIA (COFEMER)
P R E S E N T E

La Asociación Mexicana de Laboratorios Farmacéuticos, A.C. (AMELAF), organismo integrado por 52 laboratorios farmacéuticos mexicanos, como sector interesado para participar dentro del proceso de consulta pública abierto para desahogar ante la Comisión Federal de Mejora Regulatoria (COFEMER) el expediente de mejora regulatoria promovido por la Comisión Federal de Protección Contra Riesgos Sanitarios (COFEPRIS), denominado:

"Proyecto de Norma Oficial Mexicana PROY-NOM-177-SSA1-2013, que establece las pruebas y procedimientos para demostrar que un medicamento es intercambiable. Requisitos a que deben sujetarse los terceros autorizados que realicen las pruebas. Requisitos para realizar los estudios de biocomparabilidad. Requisitos a que deben sujetarse los terceros autorizados, centros de investigación o instituciones Hospitalarias que realicen las pruebas de biocomparabilidad".

En este sentido, deseamos exponer ante COFEMER nuestra opinión técnica con respecto a los documentos que obran en dicho expediente.

El jueves 15 de agosto de 2013, la COFEMER publicó tres documentos de gran trascendencia para la Industria Farmacéutica Nacional, a saber:

1.- DICTAMEN TOTAL NO FINAL, de 23 páginas, dentro del trámite de mejora regulatoria del proyecto de NOM denominado:

"Proyecto de Norma Oficial Mexicana PROY-NOM-177-SSA1-2013, que establece las pruebas y procedimientos para demostrar que un medicamento es intercambiable. Requisitos a que deben sujetarse los terceros autorizados que realicen las pruebas. Requisitos para realizar los estudios de biocomparabilidad. Requisitos a que deben sujetarse los terceros autorizados, centros de investigación o instituciones. Hospitalarias que realicen las pruebas de biocomparabilidad".

2.- Oficio de COFEPRIS, de 3 páginas, dando respuesta al Dictamen Total No Final de COFEMER.

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En la respuesta de COFEPRIS a COFECO, por lo que se refiere a la Opinión Institucional EN CONTRA de este proyecto, contenida en el documento suscrito por el Lic. Ángel López Hoher, Titular de la Unidad de Planeación, Vinculación y Asuntos Internacionales de la Comisión Federal de Competencia, de fecha 13 de agosto de 2013, se señala la objeción al requisito de hacer pruebas en territorio nacional con población mexicana, aduciendo COFECO que:

"El anteproyecto introduce barreras a la entrada para el mercado mexicano de medicamentos genéricos e intercambiables, en perjuicio del consumidor mexicano".

También aparece que en el Dictamen Total No Final, COFEMER recogió e hizo suyo este argumento de la COFECO, citado en la Pág. 15/23, ante el cual COFEPRIS proporcionó una amplia respuesta técnica bajo el encabezado:

"Referente a la solicitud de valorar los comentarios realizados por la Comisión Federal de Competencia (CFC), respecto a las razones por las que se estima imprescindible que las pruebas de intercambiabilidad y biocomparabilidad se realicen en nuestro país con población mexicana".

Es sobre este punto específico, el de que las pruebas de intercambiabilidad y biocomparabilidad de los medicamentos para consumo de los mexicanos se realicen en México con población mexicana, donde AMELAF desea enfatizar que en materia de Fármaco-Genómica, la investigación científica de los últimos años comienza a requerir que los medicamentos tengan sus pruebas de biodisponibilidad conforme al genotipo y fenotipo de las poblaciones que consumirán esos productos farmacéuticos, en virtud de las variabilidades científicamente comprobadas que existen entre diferentes grupos étnicos.

Para sustentarlo, mencionaremos aquí algunas opiniones científicas relevantes:

http://www.innsz.mx/opencms/contenido/investigacion/comiteEtica/farmacogenomica.html

INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN © 2013
12 de Marzo de 2013

Dr. José Alberto Ávila Funes

"La farmacogenómica es el término que se utiliza para describir al estudio de la contribución de las diferencias en los genes de un individuo a la variación en las respuestas a los medicamentos entre la población.

Tales diferencias en los genes tienen que ver con la producción de proteínas específicas que participan en los distintos procesos del paso de los medicamentos por el organismo, desde su absorción, su acceso al torrente sanguíneo, su

distribución en los tejidos donde se busca que tengan su efecto terapéutico, su desintegración y su posterior eliminación del cuerpo.

Una proporción de la variabilidad en la respuesta a un fármaco (tanto sus efectos terapéuticos como sus reacciones adversas) está relacionada con la variabilidad de nuestra información hereditaria. De hecho, estudios estadísticos sugieren que hasta el 60% de la variabilidad observada en la respuesta a los medicamentos en las personas está determinada por la variabilidad genética de los individuos en quienes se utilizan.

Mediante el uso de la información genética es posible identificar los cambios en el funcionamiento de diversos genes entre los individuos así como los mecanismos subyacentes y que son los responsables de las diferencias en las respuestas a los fármacos. De tal forma, la farmacogenómica tiene el potencial de maximizar la seguridad y la eficacia de los tratamientos farmacológicos en la población.

La farmacogenómica también permite a través del estudio de los genes completos de un individuo diseñar fármacos a la medida adaptados a sus características hereditarias para incrementar la efectividad y minimizar los efectos secundarios indeseables".

El nuevo modelo farmacéutico que está surgiendo entre los reguladores sanitarios, la industria farmacéutica y la medicina genómica, se encuentra sintetizado en el documento anexo, titulado:

"Farmacogenómica y la transición a un nuevo modelo farmacéutico: implicaciones económicas y de regulación".

Antonio Ramírez de Arellano Serna, Cristina Varela
Área de Farmacoeconomía, Departamento de Business Developmen, Roche Farma España

En dicho documento, una de las conclusiones de sus autores, es:

"La necesidad de adaptar el conocimiento y la práctica médica a los nuevos retos de la Farmacogenómica requiere cambios en la formación médica y una mayor implicación de los agentes reguladores traducida en provisión de guías clínicas asociadas al nuevo tipo de prescripción. Asimismo, la Farmacogenómica supone un cambio en los fundamentos actuales de los sistemas sanitarios desde un enfoque reactivo a otro predictivo".

3.- Oficio con respuesta de COFEPRIS de 4 páginas, al escrito presentado por Joseph Damond, Vicepresidente Internacional de BIOTECHNOLOGY INDUSTRY ORGANIZATION (BIO), respecto a crear la figura jurídica de la exclusividad esencial de los datos reglamentarios de los medicamentos innovadores, pidiendo a las autoridades regulatorias mexicanas que reconozcan:

"La exclusividad de datos para promover el desarrollo y comercialización de nuevas medicinas, no permitiendo la aprobación de un genérico o biosimilar que se apoye en estos datos de referencia hasta el final del plazo de protección".

La respuesta específica de COFEPRIS a dicha petición de la BIOTECHNOLOGY INDUSTRY ORGANIZATION (BIO), fue la siguiente:

"No se acepta el comentario.

Con fundamento en el artículo 33 del Reglamento de la Ley Federal sobre Metrología y Normalización, se consideró improcedente el comentario debido a que dicha propuesta no es materia que tenga atribuible regular esta Secretaría, siendo facultad de ello el Instituto Mexicano de la Propiedad Intelectual.

Aunado a esta, El RIS establece en su artículo 2 fracción XIII BIS 1. Medicamento biotecnológico biocomparable, al medicamento biotecnológico no innovador que demuestre ser biocomparable en términos de seguridad, calidad y eficacia al medicamento biotecnológico de referencia a través de las pruebas que establezca la Ley, este Reglamento y demás disposiciones aplicables;

XIII Bis 3. Medicamento biotecnológico de referencia, al medicamento biotecnológico innovador que se utilice de referencia para el registro de medicamentos biotecnológicos biocomparables y que sea reconocido como tal por la Secretaría. Cuando el medicamento biotecnológico innovador no se encuentre registrado en México, se podrá reconocer como tal a un medicamento biotecnológico biocomparable previamente registrado ante la Secretaría;

Así también la patente se encuentra regulado por el artículo 167 bis del RIS que establece:

El solicitante del registro de un medicamento alopático deberá anexar a la solicitud la documentación que demuestre que es el titular de la patente de la sustancia o ingrediente activo o que cuenta con la licencia correspondiente, ambas inscritas en el Instituto Mexicano de la Propiedad Industrial.

Alternativamente, y de acuerdo con el listado de productos establecidos en el artículo 47 bis del Reglamento de la Ley de la Propiedad Industrial, podrá

manifestar, bajo protesta de decir verdad, que cumple con las disposiciones aplicables en materia de patentes respecto a la sustancia o ingrediente activo objeto de la solicitud. En este supuesto, la Secretaría pedirá de inmediato la cooperación técnica del Instituto Mexicano de la Propiedad Industrial para que, dentro del ámbito de su competencia, éste determine a más tardar dentro de los diez días hábiles posteriores a la recepción de la petición, si se invaden derechos de patente vigentes. En caso de que el Instituto Mexicano de la Propiedad Industrial concluya que existen patentes vigentes sobre la sustancia o ingrediente activo de las que el solicitante no sea titular o licenciatario, lo informará a la Secretaría para que ésta prevenga al solicitante con el objeto de que demuestre que es titular de la patente o que cuenta con la licencia respectiva, dentro del plazo que determine la Secretaría y que no podrá ser menor a cinco días hábiles contados a partir de que haya surtido efectos la notificación. En el supuesto de que el solicitante no subsane la omisión, la Secretaría desechará la solicitud e informará al solicitante los motivos de esta determinación para que, en su caso, los dirima ante la autoridad competente. La falta de respuesta del Instituto Mexicano de la Propiedad Industrial dentro del plazo señalado se entenderá en sentido favorable al solicitante.

Sin perjuicio de lo establecido en los dos párrafos anteriores, se podrá solicitar el registro de un genérico respecto de un medicamento cuya sustancia o ingrediente activo esté protegida por una patente, con el fin de realizar los estudios, pruebas y producción experimental correspondientes, dentro de los tres años anteriores al vencimiento de la patente. En este caso, el registro sanitario se otorgará solamente al concluir la vigencia de la patente".

AMELAF apoya ese criterio de COFEPRIS, y con respecto a la pretensión de crear la figura de la "exclusividad de datos", AMELAF solicita respetuosamente a COFEPRIS y COFEMER que dicha pretensión de exclusividad de datos sea rechazada por infundada, y que, por sus efectos negativos para el país, se valore cuidadosamente el impacto adverso que tendría para los programas de salud del Estado Mexicano permitir dicha exclusividad de datos, con lo que se retrasaría el ingreso de muchos medicamentos genéricos, generando un perjuicio en la economía de los consumidores mexicanos.

Por lo antes expuesto, AMELAF expone ante ustedes que:

1.- Como resultado de la investigación científica en materia de Fármaco-Genómica, el nuevo modelo farmacéutico requiere que los medicamentos realicen sus pruebas de biodisponibilidad conforme al genotipo y fenotipo de las poblaciones que consumirán esos productos farmacéuticos, en virtud de las variabilidades científicamente comprobadas que existen en las reacciones a fármacos por parte de los diferentes grupos étnicos.

2.- Con respecto a la pretensión de introducir la figura de la "exclusividad de datos", AMELAF solicita a COFEPRIS y COFEMER que esa figura jurídica sea rechazada y que se valore cuidadosamente el impacto adverso que de aceptarla tendría para los programas de salud del Estado Mexicano.

Atentamente,

ING. RICARDO ROMAY WISBRUN
DIRECTOR EJECUTIVO

Anexos.- 7 documentos sobre Regulación Sanitaria y Fármaco-Genómica.

c.c.p. **MTRO. MIKEL A. ARRIOLA PEÑALOSA.- COMISIONADO FEDERAL PARA LA PROTECCION CONTRA RIESGOS SANITARIOS.- COFEPRIS**

LIC. ANGEL LOPEZ HOHER.- TITULAR DE LA UNIDAD.- COMISION FEDERAL DE COMPETENCIA.- UNIDAD DE PLANEACIÓN, VINCULACIÓN Y ASUNTOS INTERNACIONALES

knowledge-based economy, and to harnessing life-sciences research for health and economic development. Although the relevant policies are in place, what remains to be seen is to what extent they will be translated into concrete support for research, development and delivery of products and services in the human genomics field. Identifying the needs, encouraging local collaborations and helping to form R&D networks will go a long way towards establishing South Africa in this field.

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FURTHER INFORMATION
African Genome Education Institute (AGEI): http://www.africagenome.com
African Institute of Biomedical Science and Technology (AiBST): http://www.aibst.com
African Society of Human Genetics (AfSHG): http://www.afshg.org
Biodiversity Act: http://www.info.gov.za/gazette/acts/2004/a10-04.pdf
Burden of Disease Research Unit FAQs, Medical Research Council: http://www.mrc.ac.za/bod/faqdeath.htm
Centre for Proteomic and Genomic Research (CPGR): http://www.cpgr.org.za
Freedom to Innovate report by the African Union High Level Panel on Modern Biotechnology: http://www.nepadst.org/doclibrary/pdfs/biotech_africa_2007.pdf
Gknowmix: http://www.gknowmix.com
Lifelabs: http://www.lifelab.co.za
McLaughlin–Rotman Centre for Global Health: http://www.mrcglobal.org
National Institute of Genomic Medicine (INMEGEN), Mexico: http://www.inmegen.gob.mx
The Division of Human Genetics, University of Cape Town: http://web.uct.ac.za/depts/genetics
The Living History Project: http://www.africagenome.com/index.php?option=com_content&task=category&sectionid=4&id=13&Itemid=43
The Public Understanding of Biotechnology programme: http://www.pub.ac.za
The Skin Colour Education Project: http://www.africagenome.com/index.php?option=com_content&task=view&id=17&Itemid=39
The World Health Organisation's South Africa overview: http://www.who.int/countries/zaf/en

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SCIENCE AND SOCIETY

The next steps for genomic medicine: challenges and opportunities for the developing world

Billie-Jo Hardy, Béatrice Séguin, Federico Goodsaid, Gerardo Jimenez-Sanchez, Peter A. Singer, and Abdallah S. Daar

Abstract | This is a historical moment on the path to genomic medicine — the point at which theory is about to be translated into practice. We have previously described human genome variation studies taking place in Mexico, India, Thailand, and South Africa. Such investments into science and technology will enable these countries to embark on the path to the medical and health applications of genomics, and to benefit economically. Here we provide a perspective on the challenges and opportunities facing these and other countries in the developing world as they begin to harness genomics for the benefit of their populations.

Thanks to rapid economic development, two-thirds of the entire global economic growth last year was from the so-called emerging economies, which are predicted to grow at an average of 6.7% in 2008 compared with 1.3% in the United States, Japan and European Union[1]. In addition, emerging economies in the developing world, such as India, China and Brazil, are investing heavily in innovative science and technology (S&T) and making significant progress in the life-sciences arena, where they are increasingly protecting the subsequent intellectual property[2–4]. The situation in the poorer parts of the developing world, especially in sub-Saharan Africa, has so far been different. The proportion of gross domestic product (GPD) spent on research and development (R&D) is extremely low in sub-Saharan Africa[5], and health expenditure is less than

US$30 per capita annually[6] compared with more than $6,000 in the United States[7]. However, in terms of understanding the value of S&T for development, of investing in S&T, and of realizing the need to spend more on health, the situation might be changing. For example, African Union countries have endorsed the call to spend 1% of their GDP on S&T and have undertaken to spend more on health[8,9].

We have described several case studies in Mexico, India, Thailand and South Africa, which demonstrate how emerging economies in the developing world are investing in large-scale human genomic variation studies[10–13]. The most comprehensive programme is Mexico's National Institute for Genomic Medicine (INMEGEN), which has described in a recent publication a nine-point strategy for the adoption of genomic medicine, including: building an innovative organizational design; establishing the initial infrastructure; initiating nationwide strategic alliances; conducting R&D in genomic medicine; applying genomic technology to common health problems; reaching excellence in teaching and training programmes; supporting academic programmes in genomic medicine; addressing ethical, social and legal issues; and translating genomic knowledge into products and services[14]. For these countries that have already embarked on major genomics initiatives, establishing research institutes and conducting the research are the first steps on the path to genomic medicine. For them the major question now is how they will go from this early-phase investment towards the hoped-for health-oriented applications, and the economic benefits. For other countries that have not started genomics initiatives, the question is: what are the potential entry points? And for all developing countries, with or without current genomics initiatives, the question is: what are the challenges and opportunities along the way to the adoption of genomic medicine and to deriving economic benefit from genomics? Some challenges are common to all countries, whether economically developed or developing. Other challenges will be more specific to developing countries. Here we present a perspective on the challenges and opportunities associated with the adoption of genomic medicine, particularly in the developing world, and the need to understand the interdependent nature of efforts to develop genomic medicine globally.

Projects in the developing world

The large-scale human genomic variation projects that we have described are not the only projects of their kind taking place in emerging economies and the developing world. China was the only developing country that participated in the sequencing of the human genome. Several years later, it has a number of important initiatives related to genomics. For example, China's Beijing Institute of Genomics plans to sequence the entire genome of 100 Chinese individuals. Some relatively wealthy countries, previously unknown for their involvement in human genomics, are also investing in this field. Researchers in the Al-Mulla molecular pathology laboratory at the University of Kuwait, for example, are using haplotype mapping to study the Arab genome and to identify genomic sites linked to colorectal cancer and type II diabetes. Iran has initiated the Human Genome Diversity Project of Iran (HGDPI), with the main objective of documenting genomic diversity in the populations of Iran for anthropological purposes, and generating a database towards furthering the understanding of disease predisposition. Finally, sub-Saharan African countries other than South Africa are also investing in this field. In the year 2000, a national DNA data bank was initiated in The Gambia containing samples from ~57,000 West Africans[15,16]. More recently, a biobank and pharmacogenetics database was established in Harare, Zimbabwe, containing 1,488 samples from several ethnic sub-Saharan African populations (Nigeria, Kenya, Tanzania, Zimbabwe and South Africa)[17].

Finally, the Pharmacogenetics for Every Nation Initiative (PGENI) is also worth noting in this context as its goals include: enhancing the understanding of pharmacogenetics; building local infrastructure for pharmacogenetic studies; providing guidelines for medical prioritization; and promoting the integration of genetic information in the developing world.

Exploring potential opportunities

Countries in the developed world, such as the United Kingdom, the United States and Japan have made tremendous investments in R&D towards genomic medicine[18,19] (the UK Biobank and the US National Office of Public Health Genomics, for example). Emerging economies in the developing world that have made a similar commitment will need to consider how best to identify their next steps into genomic medicine. Thinking of these next steps might also help them identify unique niches that would give them commercial advantages. For other countries that have not yet started genomics initiatives, their entry points will depend, to some degree, on their respective life-sciences innovation infrastructure. Such entry points (see below) would need to be appropriate for their level of investment in genomics R&D and their existing health-care delivery systems.

The current trend in terms of both next steps and entry points is for countries in the developing world to collaborate in R&D with more developed nations (north–south collaborations). The Human Genome Organisation (HUGO) Pan-Asian SNP Consortium provides an example of a recent north–south R&D collaboration between Asian countries[20,21]. Lessons learned from such collaborations have contributed to the further development of international ethical guidelines for benefit sharing, ownership and R&D capacity building in human genomic research. However, increasingly there is a trend towards south–south collaborations[22], in which developing countries pool their limited resources, help each other and learn from each other's experience. For example, Mexico's significant investment in genomic research infrastructure provides other Latin-American countries lacking genomic R&D capacity the opportunity to pool their resources with Mexico, as opposed to the United States or Europe, towards the development of genomic medicine and innovative genomic medicine products for this region.

Next steps and entry points will need to be cost effective. Pharmacogenomic approaches, including diagnostics, can reduce adverse drug reactions in countries that can least afford to waste money on drugs that might not have the expected therapeutic effect. Diagnostics might be easier to develop than new drugs and vaccines, as they bypass the costly clinical trial stage and tend to have a shorter regulatory review schedule. In this respect, once the cost drops significantly, pharmacogenomic diagnostics might be an early next step or even an entry point for some developing countries. Given their access to large populations exposed to multiple infections (for example, HIV/AIDS, malaria and tuberculosis), another viable option for developing countries could be to focus genomics R&D on the host–pathogen responses for these infections.

Bioinformatics provides another potential option. The South African National Bioinformatics Institute (SANBI), for example, has developed eVOC — a software program that unifies gene expression data by facilitating a link between the genome sequence and expression phenotype information[23]. The World Health Organization-based Special Program on Tropical Diseases Research and Training runs training

programmes on bioinformatics for scientists in the developing world[24].

National genotyping projects are useful for establishing base-line profiles, which might have great benefit for subsequent studies. For example, INMEGEN has revealed significant ancestral components between populations from different regions of Mexico. Moreover, identification of unique SNPs and significant differences of functional variations related to drug metabolism suggest the need for regional approaches for the study and applications of genomic medicine in Mexico. The Indian Genome Variation (IGV) consortium recently uncovered high levels of genetic divergence between groups of Indian populations that cluster largely on the basis of ethnicity and language[25]. The study of such population groups will be useful for addressing stratification and complex study design issues[25]. Here, large collaborative efforts on R&D in fields such as pharmacogenomics, vaccinogenomics and toxicogenomics could serve as entry points for developing countries. Examples include pharmacovigilance programmes and detailed analyses of data from vaccine trials in which some but not all the human subjects respond to a particular vaccine. A well known example demonstrating that genetic factors can have a strong effect on the immune response to certain vaccines is the response to hepatitis B surface antigen (HBsAg). Up to 10% of people do not respond to HBsAg vaccination. Recent evidence suggests that although genes encoded within the major histocompatibility complex are important for this immune unresponsiveness, more than half the heritability is determined outside of this complex. Identification of these genes will help us to understand regulation of immune responses to viral proteins[26].

A potential to improve the understanding of genomics and traditional medicines through fields such as nutrigenomics provides another possible entry point that offers these countries an intellectual property advantage. Traditional medicine is well established in China and India, where a memorandum has recently been signed to further the understanding of their respective traditional medicine sectors[27]. Whether genomics can add any value to such endeavours remains to be seen.

Although limited, private sector firms in developing countries have also begun to leverage the opportunities in genomic medicine, identifying possible entry points and using unique resources. Avesthagen Ltd, an Indian-owned life-sciences company, has a large-scale genotyping project of the Parsi population in India[28]. They predict an initial market in translational medicine, such as genomic medicine, with an eventual foray into early diagnosis, pre-symptomatic and life-long treatment through a combined offering of wellness products (for example, nutrigenomics) and personalized health care. In other countries, such as South Africa and Thailand, a few innovative firms are considering targeting the medical tourism market[12,13].

Finally, additional possible entry points might involve anthropology or human history and migration studies, as part of establishing a base-line of data for possible health applications. This, for example, was the original impetus for the HUGO Pan-Asian SNP Consortium[20,21]. Other countries might become involved as a result of participating in the HapMap project, as did Nigeria[29,30].

Challenges

Genomic research platforms in emerging economies and developing countries will be faced by a number of similar challenges as they become established and proceed towards the adoption of genomic medicine in their respective countries. These challenges are: the current lack of skilled human resources; ensuring sustainable funding and political will; sourcing alternative funding; improving collaboration within the public research sector as well as between the public research sector and the private sector; developing opportunities for south–south and north–south collaboration; improving the commercialization infrastructure in both the public and private sector; developing and improving the existing regulatory infrastructure; developing a health-care infrastructure that can address access and delivery issues of genomic medicine; training health-care workers; and engaging with the public to improve awareness and participation[10–14]. A number of these challenges are local and can be addressed as such by and in individual countries. Others, such as establishing international R&D collaborations in genomics research and the need to address the lack of harmonized regulatory infrastructure for genomic medicine, require collaborative efforts on an international scale to address them.

Internationally, issues that rapidly need to be addressed for productive and equitable collaborations include data and sample sharing, research capacity building in developing countries, and rules and guidelines for building and using international repositories containing long-term treatment outcomes in both developed and developing nations. These issues are often not straightforward to address. For example, data and sample sharing in many developed countries have traditionally focused upon consent and the concerns associated with privacy and confidentiality[31]. But in international collaborations, in addition to these concerns, considerations will also have to be given to the sovereign nature of the data and of samples sourced in emerging economies and developing countries[32]. The Public Population Project in Genomics (P3G), an international consortium that aims to build the necessary collaborative infrastructure between institutes in order to foster interoperability on the research level[33], provides one example of how international consortia can contribute to addressing these challenges. It is important to think about such issues early because the need for large-scale collaborative research is becoming more pressing as R&D in genomic medicine advances. These studies will require data comparisons and validation in large sample sets across different populations[34], which is one reason the European Science Foundation has recommended that European biobanking initiatives harmonize their efforts to achieve maximal benefit[35].

Collaborative efforts will also be necessary to address policy issues related to translation of genomic research and applications for population health. An example is the Genome-based Research and Population Health International Network (GRaPH-Int), a global collaborative network that fosters dialogue, research, education and training, and communication and stakeholder engagement, with the aim of establishing public policies, programmes and services in public health genomics. These types of consortia will be necessary for integrating approaches to genomic medicine and accelerating global consensus-building towards the development of international standards, the regulation of genetic testing and consumer genomics, and accessibility of genetic data and associated information.

Broader issues that require consideration include the integration of genetic information into public health decision-making, guidelines for medical prioritization, intellectual property regimes, and the association between regulatory bodies and health technology assessment bodies. For instance, forward-looking intellectual property regimes will need to be developed that can be used by countries, depending on the stage of economic and scientific development, to facilitate access to health products, scientific capacity building or economic profit. There is also a great need to build capacity in

technology transfer in many developing countries.

One of the major challenges in the application of genomic medicine in emerging economies and developing countries involves the limited, or even absent, regulatory infrastructure. Some of these countries may have limited capacity to regulate traditional drugs and diagnostics, and will need to build capacity for these and for emerging genomic medicine products. Furthermore, regulatory capacity in many developing countries will need to encompass the work of ministries of health, science and technology, industry, commerce, natural resources, the judiciary and legislative bodies, as well as of drug licensing agencies. Developing countries might benefit from the experience of developed countries that are currently drafting the necessary guidelines to address unique emerging issues associated with the regulation of genomic medicine products[36]. In the developed world, these issues are being addressed on a national scale by their regulatory agencies and on an international scale through the International Conference on Harmonization (ICH)[37]. The inclusion of emerging or developing countries in the ICH harmonization of guidelines associated with genomic medicine, as well as in other consortia that can help to improve regulatory capacity, will provide a concrete opportunity to improve the application of genomic medicine to global health.

Genomic medicine is redefining how both developed and developing countries need to work together in the application of new knowledge to improve public health. The intrinsic value of information in the human genome is associated less with national boundaries and income than with how comprehensive this information is, and is strongly dependent on the information from many individual genomes and from many individual countries. Data acquired from the genotyping and sequencing projects in emerging and developing countries summarized above help to bridge the gaps in the application of genomic medicine between developing and developed countries. Access to these data throughout the world will be crucial for the identification of novel biomarkers of drug safety and efficacy. Whereas the generation of information from the human genome requires a global effort, its application will require development of therapeutic, diagnostic and other applications and products aimed at sub-populations and individuals. The most important benefits will probably be in the use of genomics knowledge to prevent diseases and promote health. At an individual level, and perhaps a sub-population level, the benefits will depend on how genomics is integrated in health systems. To achieve these aims of genomic medicine on a larger scale, public health systems in countries throughout the world will need to integrate pharmacogenomic data from their citizens and will need to use these data effectively for the optimal allocation of diagnostic and therapeutic resources, to improve health education of their publics. This will help their public to change their health-related behaviour, to educate health professionals, and to re-engineer health-care systems more towards prevention and health promotion.

In terms of the benefits of S&T generally, the current trend to develop knowledge, skills and products in the economically and scientifically more developed countries and then struggle to make these available to the less scientifically developed and poorer countries is not sustainable in the long run. This is why so many developing countries, especially the emerging economies, are focusing more on local innovation, invention and commercialization to break the cycle of dependency. To go 'from the lab to the village' through discovery to product development to commercialization by, and in, developing countries will require that science, business and capital be brought together creatively. One proposal involves creating 'convergence centres', which are an evolution beyond science parks and incubators, aimed specifically at enhancing opportunities for knowledge sharing and rapid innovation, and have a focus on product development and commercialization[38].

Conclusions

The existing initiatives in emerging economies in the developing world, which range from databases run by research networks to comprehensive national institutes with public health mandates, serve as models for how to invest in innovative S&T towards development. Such initiatives can strengthen local research infrastructure and local intellectual property regimes, address local health needs and reduce health-care costs, thus improving local health equity. How these countries choose to capture these benefits early on will depend, in part, on the type of entry points they opt for, which in turn will depend on the level of development of their research and health infrastructure. There is much to be learnt from the experience and examples of India, Mexico, Thailand and South Africa.

There are, however, significant challenges to be addressed before the adoption of genomic medicine, and although these are for the most part being experienced by all countries, the challenges are more pronounced for developing countries. There is therefore a need to strengthen existing collaborative efforts, especially south–south collaborations, which in the long run might be more sustainable. Current and future initiatives and investments in R&D capacity will further enable countries in the developing world to participate as equal R&D partners with more developed countries, instead of merely facilitating access to local biological resources.

In terms of international collaborations that would add value to all countries, at a recent workshop in Mexico City (see Emerging Regulatory Issues in Genomic Medicine conference overview) experts in the emerging issues in genomic medicine from both the developed and developing world discussed a number of ways forward. They included the creation of an information clearing-house, a virtual network of interested parties, phenotypic and genotypic databases associated with drug development, an international consortium for biomarker development with real involvement of emerging economies and developing countries, and methods of international harmonization of genomic product regulation. Many in the developing world are excited with the prospects of genomic medicine. We are at the crossroads between theory and practice. It is important now that we all think seriously about the challenges and opportunities lying ahead.

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FURTHER INFORMATION

Al-Mulla molecular pathology laboratory, University of Kuwait: http://www.al-mulla.org
Avesthagen Ltd: http://www.avesthagen.com
Beijing Institute of Genomics, China: http://www.big.ac.cn/big/english/index.jsp
Emerging Regulatory Issues in Genomic Medicine conference overview: http://regulatory.inmegen.gob.mx/english/home.html
European Science Foundation: http://www.esf.org
eVOC software program: http://www.evocontology.org
Genome-based Research and Population Health International Network (GRaPH-Int): http://www.graphint.org/ver2
Human Genome Diversity Project of Iran (HGDPI): http://www.nrcgeb.ac.ir/Human%20Genom%20Diver.html
International Conference on Harmonization (ICH): http://www.ich.org/cache/compo/276-254-1.html
McLaughlin–Rotman Centre for Global Health: http://www.mrcglobal.org
National Institute for Genomic Medicine (INMEGEN), Mexico: http://www.inmegen.gob.mx
Pharmacogenetics for Every Nation Initiative (PGENI): http://pgeni.unc.edu
South African National Bioinformatics Institute (SANBI): http://www.sanbi.ac.za
The Public Population Project in Genomics (P3G): http://www.p3gconsortium.org
UK Biobank: http://www.ukbiobank.ac.uk
US National Office of Public Health Genomics: http://www.cdc.gov/genomics/default.htm

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Association of the genetic marker for abacavir hypersensitivity *HLA-B*5701* with *HCP5* rs2395029 in Mexican Mestizos

Prospective screening for *HLA-B*5701* decreases or abolishes abacavir hypersensitivity reaction. In Caucasians, the HLA complex protein 5 gene (*HCP5*) rs2395029(G) allele is in complete linkage disequilibrium (LD) with *HLA-B*5701* ($r^2 = 1$). **Aim:** To assess the frequency of *HLA-B*5701* and its LD with *HCP5* rs2395029(G) allele, to extend our knowledge of genetic variants that are of critical relevance for the development of pharmacogenetics in Mexico. **Materials & methods:** We genotyped 300 Mexican Mestizos from the Mexican Genome Diversity Project. *HLA-B*5701* genotyping was performed using a DNA sequencing method. *HCP5* rs2395029 was genotyped using a custom TaqMan® SNP genotyping assay and confirmed by direct sequencing. Genotypes for 14 SNPs in the *HCP5* region were retrieved from the Mexican Genome Diversity Project database for LD analysis. **Results:** *HLA-B*5701* carrier frequency was 2% and the allelic frequency was 0.010. Haplotype analysis revealed that *HLA-B*5701* and the *HCP5* rs2395029(G) allele are in complete LD ($r^2 = 1$) in this Mexican Mestizos sample. **Conclusion:** It is feasible to have a pharmacogenetic program based on *HCP5* rs2395029 genotyping as a screening tool with confirmation of *HLA-B*5701* carriage by sequenciation, to prevent abacavir hypersensitivity reaction in Mexican patients before initiating abacavir therapy.

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KEYWORDS: abacavir *HCP5* *HLA-B*5701* hypersensitivity pharmacogenetics

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Abacavir is a nucleoside reverse-transcriptase inhibitor antiretroviral indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adult and pediatric patients. Abacavir is associated with potentially serious hypersensitivity reactions in 3–9% of Caucasian patients that require immediate and permanent discontinuation of the drug [1]. It has been shown that carriers of the allele 5701 of the *HLA* locus B (*HLA-B*5701*) are at high risk for developing abacavir hypersensitivity reaction (AHR) [2–4] and that prospective genetic screening for *HLA-B*5701* greatly diminishes or abolishes the occurrence of AHR [5–7].

In July 2008, the US FDA approved a label change for abacavir (Ziagen) to include a boxed warning indicating that patients who carry the *HLA-B*5701* allele are at high risk for experiencing a hypersensitivity reaction to abacavir and a recommendation to screen for *HLA-B*5701* prior to the initiation of abacavir therapy [101].

In Caucasians, rs2395029 (c.335T>G) in the HLA complex protein 5 gene (*HCP5*), located 100 kb centromeric of *HLA-B*, is in complete linkage disequilibrium (LD) with *HLA-B*5701* (r^2=1) [8–10]. Further studies have identified recombination events at multiple sites within the MHC [11], revealing a high but incomplete LD between *HLA-B*5701* and *HCP5* rs2395029 [11–13].

Mexico's population is mainly composed of Mestizos, who, as with other Latin American populations, are a recently admixed population composed of Amerindian, European and, to a lesser extent, African ancestries.

The aim of our study was to asses the prevalence of *HLA-B*5701*, *HCP5* rs2395029(G) and their LD pattern in the Mexican Mestizo population by analyzing the samples from the Mexican Genome Diversity Project (MGDP), to extend our knowledge of genetic variants of critical relevance for the development of pharmacogenetics in Mexico.

Materials & methods

The panel of 300 Mexican Mestizo samples from the MGDP consists of nonrelated, self-identified Mestizo individuals having four grandparents not self-recognized as recent immigrants from six states in geographically distant regions of Mexico [14]: Sonora and Zacatecas in the north, Guanajuato in the central region, Guerrero in the central/Pacific region, Veracruz in the central/Gulf region and Yucatan in southwest Mexico. From those, 268 samples were available for genotyping.

*HLA-B*5701* genotyping was performed by DNA sequencing using the AlleleSEQR HLA-B PCR/sequencing Kit (Abbott Laboratories, IL, USA) and the genetic analyzer 3130×L

Table 1. List of 15 haplotypes found in the best reconstruction with PHASE V2.1.

Haplotype content	Count	Percentage
113114241224333	198	33.0
112124241422333	147	24.5
113124241222333	96	16.0
113114141224333	56	9.3
133324221222131	28	4.7
113114241214333	18	3.0
133322221222331	16	2.7
133324241222331	15	2.5
133314241224333	13	2.2
213114242224333[†]	6	1.0
133324221222111	3	0.5
113314241224333	1	0.2
113114241224331	1	0.2
113112241224333	1	0.2
113124241222331	1	0.2

[†]*Bold numbers at positions 1 and 9, respectively, indicate that the haplotype contains both the rs dummy allele, which represents the presence of* HLA-B*5701 *and G allele of* HCP5 rs2395029.

(Applied Biosystems, CA, USA). Sequence analysis was carried out with Assign-SBT software (Conexio Genomics, Freemantle, Australia). To genotype *HCP5* rs2395029, we used a custom TaqMan® SNP genotyping assay designed with File Builder 3.0 (Applied Biosystems) and TaqMan GT Master Mix (Applied Biosystems). To confirm the genotype determined by the allelic discrimination, 15% of the samples were sequenced using locus specific primers (forward: TACCCTCATTGTGTGACAGCA, reverse: GTCGTGGGATTTTGCACT) and the SNP's PCR amplification protocol, which is available at [102]. We amplified a 252-bp fragment containing the rs2395029 SNP. Amplicons were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were purified by BigDye XTerminator® (Applied Biosystems) prior to loading on the ABI 3730xL DNA Analyzer. ABI files were analyzed with the Lasergene SeqMan sequence analysis software (DNASTAR, WI, USA).

The MGDP database contains genotypes from 300 Mexican Mestizo samples that were analyzed with three different platforms with a total of nearly 1.4 million SNPs, including rs2395029. Genotypes of *HCP5* rs2395029 and 13 other SNPs in the *HCP5* region (Supplementary Table 1, www.future-medicine.com/doi/suppl/10.2217/pgs.11.31) were retrieved from the MGDP database. We created a dummy variable to represent the *HLA-B*5701* genotype. The variant allele represented the presence of *HLA-B*5701*, while the wild allele represented the absence of *HLA-B*5701*. Retrieved genotypes and the dummy variable were used for imputation of missing *HLA-B*5701* genotypes (n = 50) by haplotype reconstruction with PHASE software version 2.1 [15]. Phased genotypes and the dummy rs were included for LD analysis with the Haploview software [16]. Individual ancestry estimates were calculated using STRUCTURE as previously described [14].

Results & discussion

Of the original 300 samples in the MGDP, 268 samples were available for testing and were used

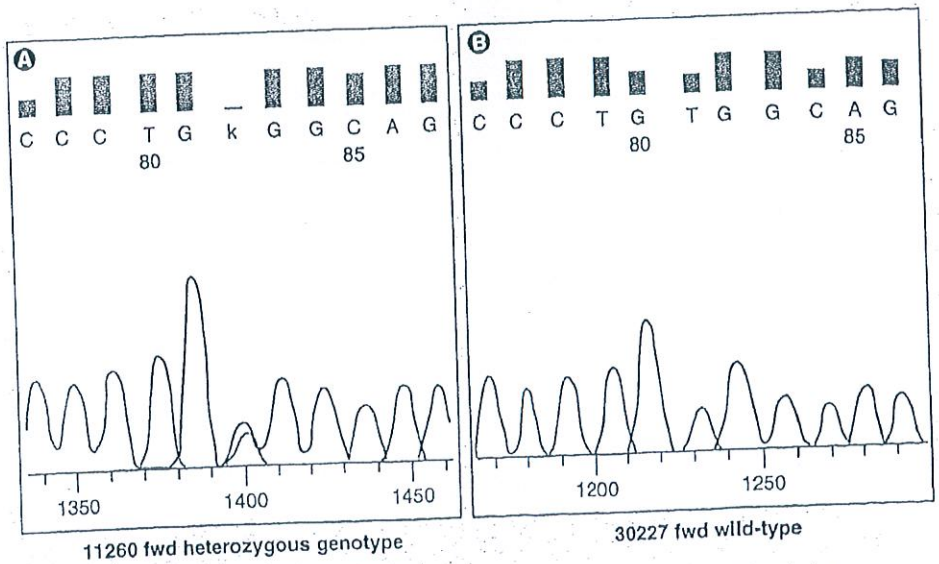

Figure 1. Typical sequencing results of (A) the heterozygous *HCP5* rs2395029 and (B) wild-type genotypes.

to characterize the prevalence of *HLA-B*5701* and *HCP5* rs2395029. Of these, 250 samples were successfully genotyped for *HLA-B*5701* and 18 samples could not be genotyped because of DNA degradation. *HCP5* rs2395029 was successfully genotyped in all 268 samples. We found six individuals who were heterozygote carriers of *HLA-B*5701* and were also heterozygote carriers of the G allele of rs2395029. There was complete agreement between the rs2395029 genotype by allelic discrimination and the genotype retrieved from the MGDP database. Haplotype reconstruction with PHASE software found 15 different haplotypes, only one of which had both the rs dummy allele representing the presence of *HLA-B*5701* and the G allele of *HCP5* rs2395029 (Table 1). The missing *HLA-B*5701* genotypes were imputed by haplotype reconstruction. None of the imputed genotypes carried *HLA-B*5701*; thus, only six out 300 individuals were heterozygote carriers for both *HLA-B*5701* and the G allele of *HCP5* rs2395029. Sequenciation confirmed the six heterozygote genotypes as well as 44 randomly chosen wild-type genotypes. Typical sequencing results of the heterozygous *HCP5* rs2395029 and wild-type are compared in Figure 1. The carrier frequency for *HLA-B*5701* and the *HCP5* rs2395029 G allele was 2.0%; whereas, their allelic frequency was 0.010 (Table 2). The LD analysis of 600 phased haplotypes showed that *HLA-B*5701* and *HCP5* rs2395029(G) allele are in complete LD (r^2=1) (Figure 2). Ancestry analysis of *HLA-B*5701* carriers showed that their average ancestry estimates for European, Amerindian, African and East Asian contributions (33, 63, 3.5 and 0.05%, respectively) are similar to the mean contributions observed in the 300 Mexican Mestizos analyzed (0.418 ± 0.155, 0.552 ± 0.154, 0.018 ± 0.035 and 0.012 ± 0.018, respectively) [14]. European contribution in *HLA-B*5701* carriers was not significantly different (t-test, p = 0.125) from the mean European contribution.

The aim of our study was to asses the prevalence of *HLA-B*5701*, *HCP5* rs2395029 and their LD pattern in the Mexican Mestizo population, to extend our knowledge of genetic variants of critical relevance for the development of pharmacogenomics in Mexico. We found a 2.0% carrier and 0.010 allelic frequencies that are similar to those previously reported in Hispanic subjects living in the USA [17]. Mexican Mestizos have a lower frequency of *HLA-B*5701* compared with the 5–8% reported in Caucasians, but a higher frequency than the 0% reported in China, Japan [18] and Korea [19], which is most likely related to our Caucasian and Amerindian ancestries.

Table 2. *HLA-B*5701* and *HCP5* rs2395029 results in 600 samples (300 individuals).

SNP	*HLA-B*5701*	Other *HLA-B* alleles
Rs2395029 (G)	6	0
Rs2395029 (T)	0	594

Prevalence 2.0%, allele frequency 0.010.

In this population, *HLA-B*5701* and *HCP5* rs2395029(G) alleles were in complete LD (r^2 = 1); this should be interpreted cautiously, as previous reports demonstrate that the LD is high but not complete [11–13]. We did not observe individuals that were *HCP5* rs2395029(G) positive and *HLA-B*5701* negative, this may be due to the relatively low *HLA-B*5701* allele frequency observed in this study and the small number of individuals in the MGDP panel.

There are various reports of successful implementation of prospective *HLA-B*5701* genetic screening programs for patients initiating abacavir therapy. These programs resulted in significant decrease of AHR [5–7,20]. The sequencing-based genotyping method is the gold standard in screening for *HLA-B*5701*; however, its use is limited because it is labor intensive, costly and requires an instrument not easily available in clinical laboratories. The PCR sequence-specific primer (SSP) assay is a reliable alternative for *HLA B*5701* genotyping. This method is currently used by several laboratories for *HLA-B*5701* screening in patients initiating abacavir [21].

Colombo and Rodríguez-Nóvoa showed that in HIV patients, the *HCP5* rs2395029 had a positive predictive value of 100%; however, the negative predictive value for carriage of *HLA-B*5701* was 94 and 93%, respectively, due to the presence of *HLA-B*5701* negative but *HCP5* rs2395029(G) positive individuals, relying exclusively on *HCP5* rs2395029, those individuals would be unnecessarily excluded from abacavir therapy. Both authors agree that *HCP5* rs2395029 could be an alternative in situations where *HLA-B*5701* testing is not easily available [12,13].

Mexico is the Latin American country with the second largest number of people living with HIV. The 2009 statistics on HIV–AIDS from the National Center of Prevention and Control of HIV-AIDS (CENSIDA) from the Secretary of Health reports that in November 2009, there were 220,000 people living in Mexico with HIV [103]. Based on UNAIDS

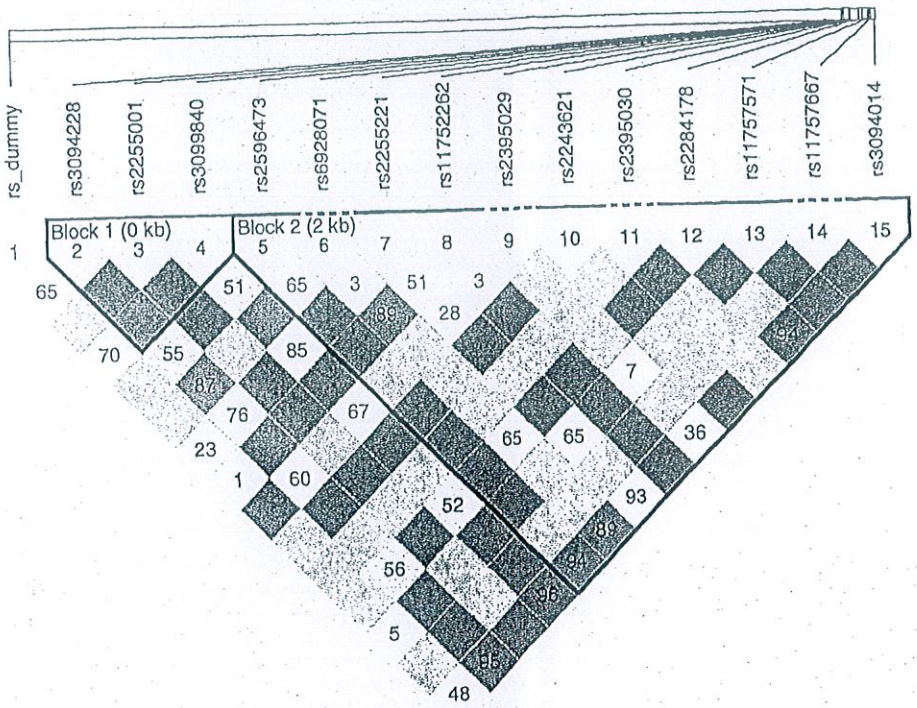

Figure 2. Linkage disequilibrium plot for rs dummy, which represents the presence of *HLA-B*5701* and 14 SNPs in the *HCP5* region. Linkage disequilibrium analysis was performed with the Haploview software by confidence interval method. Linkage disequilibrium (r^2) between rs dummy and rs2395029 is = 1.

Mexico Epidemiological Fact Sheet on HIV and AIDS 2008 Update [104], we estimate that 84,000 patients need antiretroviral treatment; however, only approximately 47,000 are receiving it. There are no published data on the number of patients receiving abacavir in Mexico, but using the figure reported by Lalonde in Canada [22], we estimate that 15,600 patients (a third) have been exposed to abacavir and have thus been at risk of presenting an AHR. *HLA-B*5701* is one of the best examples of the clinical utility of a genetic marker to identify individuals at risk for an adverse drug reaction. The clinical application of *HLA-B*5701* as a genetic marker to reduce the number of cases of AHR has been comprehensively described [20].

Studies evaluating the cost–benefit ratio of prospective *HLA-B*5701* genetic screening showed that the benefit will depend on the cost of the treatment, the cost of the genetic test and the prevalence of the genetic marker [23,24]. Our study reveals the prevalence of *HLA-B*5701* in a Mexican Mestizo population and the potential use of a single SNP genotyping method as a low cost screening tool that, together with epidemiological data, can be used for future, cost-effective, public interventions.

Single SNP genotyping is an easier method with a much lower cost compared with sequencing or SSP. This lower cost could facilitate the implementation of prospective genetic screening programs for AHR, especially in countries such as Mexico and other Latin American countries with limited access for sequencing-based HLA typing or limited resources. However, samples positive for *HCP5* rs2395029(G) allele must be confirmed as carriers of *HLA-B*5701* by either a sequencing or SSP method because of the high but not complete LD between those two genetic markers. The clinical utility of such a program must be confirmed by a clinical trial in Mexican Mestizo HIV patients that will initiate treatment with abacavir.

In conclusion, the prevalence of *HLA-B*5701* carriers in the Mexican Mestizo population is 2.0%, and the allelic frequency of *HLA-B*5701* is 0.010. There was a complete LD between *HLA-B*5701* and the *HCP5* rs2395029(G) allele in the population studied. We estimate that in Mexico there is a significant number of people exposed to abacavir, according to the prevalence of *HLA-B*5701* found in this study and the estimated number of AHR that may have occurred; we believe that it would be of

clinical relevance to have a pharmacogenetic program for Mexican patients who will initiate abacavir therapy, using *HCP5* rs2395029(G) as a screening tool and confirmation of *HLA-B*5701* carriage by either sequencing or SSP methods. A clinical trial in Mexican Mestizo HIV patients to confirm the clinical relevance is needed. Considering that a single SNP genotyping will be more accessible and affordable, the implementation will be favored, increasing patient's safety. It is important to note that genetic screening to prevent AHR should never substitute for clinical vigilance in patients who start abacavir treatment.

This information, novel in Latin America, could also be useful for other populations that share ancestral history with the Mexican Mestizo population. The present study is also an example of the use of the MGDP database for pharmacogenomic studies in the Mexican Mestizo population.

Future perspective

A single SNP genotyping together with a confirmation method will favor the implementation of pharmacogenetic programs through accessible and affordable tests that will increase patient's safety. Pharmacogenetic tests will be increasingly available at clinical laboratories and will enable the practice of personalized medicine.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Introduction

- Abacavir is an antiretroviral indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. It is associated with potentially serious hypersensitivity reactions in 3–9% of Caucasian patients.
- Carriers of *HLA-B*5701* are at high risk for developing abacavir hypersensitivity reaction (AHR). Prospective genetic screening for *HLA-B*5701* greatly diminishes or abolishes the occurrence of AHR.
- In Caucasians, *HCP5* rs2395029(G) is in high but not complete linkage disequilibrium (LD) with *HLA-B*5701*.

Results & conclusion

- Analysis of the 300 samples from the Mexican Genome Diversity Project revealed a prevalence of *HLA-B*5701* carriers of 2.0%, and the allele frequency is 0.010. There was a complete LD between *HLA-B*5701* and the *HCP5* rs2395029(G) allele in this population studied.
- It would be of clinical relevance to have a prospective pharmacogenetic program implemented for Mexican patients who will initiate abacavir therapy, using *HCP5* rs2395029(G) as a screening tool and confirmation of *HLA-B*5701* status by either a sequencing or PCR sequence-specific primer methods. The clinical utility of such a program must be confirmed by a clinical trial in Mexican Mestizo HIV patients that will initiate treatment with abacavir. Genetic screening to prevent AHR should never substitute for clinical vigilance in patients who start abacavir treatment.

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Hypercontrols in Genotype-Phenotype Analysis Reveal Ancestral Haplotypes Associated With Essential Hypertension

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Abstract—The angiotensinogen gene locus has been associated with essential hypertension in most populations analyzed to date. Increased plasma angiotensinogen levels have been proposed as an underlying cause of essential hypertension in whites; however, differences in the genetic regulation of plasma angiotensinogen levels have also been reported for other populations. The aim of this study was to analyze the relationship between angiotensinogen gene polymorphisms and haplotypes with plasma angiotensinogen levels and the risk of essential hypertension in the Mexican population. We genotyped 9 angiotensinogen gene polymorphisms in 706 individuals. Four polymorphisms, A-6, C4072, C6309, and G12775, were associated with increased risk, and the strongest association was found for the C6309 allele (χ^2=23.9; P=0.0000009), which resulted in an odds ratio of 3.0 (95% CI: 1.8–4.9; P=0.000006) in the recessive model. Two polymorphisms, A-20C (P=0.003) and C3389T (P=0.0001), were associated with increased plasma angiotensinogen levels but did not show association with essential hypertension. The haplotypes H1 (χ^2=8.1; P=0.004) and H5 (χ^2=5.1; P=0.02) were associated with essential hypertension. Using phylogenetic analysis, we found that haplotypes 1 and 5 are the human ancestral haplotypes. Our results suggest that the positive association between angiotensinogen gene polymorphisms and haplotypes with essential hypertension is not simply explained by an increase in plasma angiotensinogen concentration. Complex interactions between risk alleles suggest that these haplotypes act as "superalleles." **(*Hypertension*. 2012;59:00-00.) • Online Data Supplement**

Key Words: angiotensinogen ■ genetics ■ haplotypes ■ hypertension ■ population

Essential hypertension (EH) is a complex disease that results from an interaction between genes and environmental factors. It has been stated that 40% of hypertension is attributable to genetics.[1] The genes involved in the renin-angiotensin system have been suggested as candidate genes for EH because they play an important role in the regulation of blood pressure (BP).[2] Genetic linkage studies have demonstrated that there is a relationship between the angiotensinogen gene (*AGT*) locus and EH in white, African-Caribbean, Asian, and Mexican-American populations.[3–6] However, association studies were unable to replicate the influence of these genetic polymorphisms in the risk to EH across other populations. For example, the polymorphisms C4072T (T235M) has been associated with EH in white populations,[3,7–9] but it has been reported as borderline in a Japanese population[10] and was not associated in Arabian and African-derived populations.[4,11,12] Increased plasma levels of angiotensinogen (AGT) produced by increased levels of A-6G–induced transcription, which is in almost complete linkage disequilibrium with C4072T (T235M), has been proposed as a causal mechanism.[13] Nevertheless, the C4072T (T235M) polymorphism was not significantly associated with plasma AGT levels or with EH risk in either African-Caribbean or Mexican-American families.[4,6] Moreover, an analysis of the *AGT* polymorphisms that affect plasma AGT levels in a Japanese population showed that C4072T (T235M) did not alter the trait.[14] These facts suggest that population genetic diversity plays an important role in the control of intermediate traits, such as plasma AGT levels, and the risk for developing EH. In the case of the *AGT*, differences in the linkage disequilibrium pattern, number, and frequencies of haplotype blocks among populations

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with distinct ancestry have been described, further supporting the idea that population differences influence the association results.[15,16]

Mexican Mestizos, like other Latino populations, are a recently admixed population conformed by Amerindian, European, and, to a lesser extent, African ancestries. Previously, we evaluated genetic diversity, linkage disequilibrium patterns, and extent of haplotype sharing using genome-wide data from Mexicans Mestizos. Ancestry was evaluated by comparing 1 Mexican Amerindian group and data from the HapMap.[17]

The aim of this study was to analyze the effect of 9 polymorphisms across *AGT* on plasma AGT levels, and the risk of EH in an urban sample of the Mexican Mestizo population. To address this issue, we performed an association study and haplotype analysis. We decided to sample individuals >65 years of age as controls (hypercontrols) to limit false-negative subjects in the analysis.

Methods

Study Participants and Phenotype Definition

Patients were recruited from the outpatient clinic of the Central North Hospital and Central Military Hospital in Mexico City from February 2008 through July 2010. The patients mainly resided in the Mexico City metropolitan area, and they were self-defined as Mexican Mestizo. The diagnosis of EH was established in patients who lacked a secondary cause of hypertension after a complete clinical and biochemical examination. The hypertensive subjects included in this study met the following criteria, ≥30 years old, have a previous diagnosis of hypertension by a physician, and use a prescription antihypertensive medication and/or have a systolic BP ≥145 mm Hg and/or a mean diastolic BP ≥95 mm Hg during interview. For further information on exclusion criteria, BP measurement methods, and power statistics, see the online-only Data Supplement.

Genotyping

Genomic DNA was obtained from peripheral blood using the Qiagen Maxi-kit. We genotyped the following 9 single nucleotide polymorphisms (SNPs) at the *AGT* locus using 5′ nuclease TaqMan assays (ABI Prism 7900HT Fast RT-PCR System; Applied Biosystems, Foster City, CA): C-532T (rs5046), G-217A (rs5049), A-20C (rs5050), A-6G (rs5051), C3389T-T174M (rs4762), C4072T-T235M (rs699), C6309T (rs2493132), C11535A (rs7079), and G12775A (rs9431580). These SNPs are distributed over 13 kb across the *AGT* locus. All of the samples included had genotype call rates >95% per SNP.

Assay Procedures

Plasma AGT levels were quantified using a sandwich ELISA method as described previously.[18]

Statistical Analysis

For single-marker analysis, the allele frequencies of the 9 SNPs were estimated using direct allele counting. The Hardy-Weinberg equilibrium was calculated for each SNP using χ^2 with 1 degree of freedom. Comparisons between cases and controls for each SNP were tested using a χ^2 test with the Pearson P value under the allelic and dominant models. Odds ratios (ORs) and 95% CIs were calculated to estimate the relative hypertension risk associated with the *AGT* polymorphisms.

Single SNP association analysis was performed via a pairwise analysis between genotypes and plasma AGT levels and reported as the mean and SD; we used the AA genotype as a reference group and the other genotypes, AB and BB, for comparison. We used the Student t test to identify differences between genotypes, and a 2-tailed P value <0.005 was considered to be statistically significant after adjusting for 9 comparisons (Bonferroni correction). Potential associations between the set of haplotypes and EH status were examined by using an omnibus likelihood ratio test with permutation-based hypothesis testing procedure as implemented in HAPLOVIEW software.[19]

The haplotype inference and its effects on plasma AGT levels were evaluated using the maximum-likelihood model with the Stochastic-EM algorithm as implemented in the THESIAS software.[20] Also, Bonferroni correction was used to control for multiple testing. The genetic distance between haplotypes was estimated by the Kimura method, and a neighbor-joining network was constructed using the PHYLIP 3.6 software.[21]

The power to detect association between SNPs and EH was estimated using the Genetic Power Calculator.[22] The case-control study with 706 subjects reached 85% and 89% statistical power at=0.05 for the allelic and dominant models, respectively. The statistical power calculation for trait association was established based on the differences in means of plasma AGT levels between genotypes, using Stata 10.1 (Stata Corp). This data set achieves 99% and 86% power at 2-tailed 0.05 for C3389T (T174M) and T-20G, respectively. For further information on statistical analysis detail, see the online-only Data Supplement.

Results

Population Characteristics

The demographic information and the distribution of characteristics related to obesity and hypertension in the sample population are summarized in Table S1, available in the online-only Data Supplement. There was a difference in age between cases and controls, because the controls were deliberately selected to include subjects >65 years of age, with the hypothesis that these healthy controls have minimal genetic risk for developing EH. Sex proportions were 0.66 females and 0.34 males for cases and 0.47 females and 0.53 males for controls. Genotype distribution analysis showed no significant differences by sex (data not shown). This is consistent with the logistic regression analysis using sex as a covariate, where risk to EH by associated alleles was not affected. We analyzed a potential population stratification using 8 ancestry informative markers to distinguish between Amerindian and European ancestral components and used the principal component analysis implement in Eigensoft software to detect significant deviation in cases and controls (Figure S1, available in the online-only Data Supplement). Our results showed Fst (measure of genetic structure) between cases and controls at 0.006, with SE=0.003. This led us to conclude that there is no evidence of stratification between cases and controls in the population studied.

Association Analysis Between SNPs and EH Risk

An association analysis was performed with 502 cases and 204 hypercontrols. No deviation from Hardy-Weinberg equilibrium in the 9 SNPs was observed. Four *AGT* alleles, A-6, C4072, C6309, and G12775, were found to be associated with increased EH risk (Table S2). The strongest association was found for the C6309 allele (χ^2=23.9; P=0.0000009). This allele produced the maximum OR in the recessive model (OR: 3.0; 95% CI: 1.8–4.9; P=0.000006) with allele positivity (OR: 2.1; 95% CI: 1.4–3.2; P=0.0008; Table S3). After 10 000 permutations, the associations remained significant, at P=0.0003, P=0.001, P=0.002, and P=0.002 for C6309, A-6, C4072, and G12775, respectively. In addition, the logistic regression analysis showed that the alleles A-6, C4072, C6309, and G12775 were independent risk factors for EH after adjustment for age, sex, body mass index (BMI), and plasma AGT (Figure S2).

Table 1. Common *AGT* Haplotypes and Plasma AGT Levels in Cases and Controls

Haplotypes													
	−532	−217	−20	−6	3889	4072	6309	11535	12775	Estimation Cases (95% CI)	*P**	Estimation Controls (95% CI)	*P*
H1	C	G	A	A	C	C	C	C	G	Intercept −2.8		Intercept −2.6	
H2	C	G	A	G	C	T	T	A	A	0.20 (−2.3 to 1.9)	0.85	2.1 (−3.4 to 6.6)	0.15
H3	C	G	C	A	T	C	C	C	G	−3.6 (−6.3 to −0.9)	0.008	−4.3 (−7.6 to −1.0)	0.01
H4	C	G	A	G	C	T	T	C	A	1.4 (−1.3 to 4.0)	0.30	1.6 (−3.4 to 6.6)	0.53
H5	C	G	C	A	C	C	C	C	G	1.3 (−1.5 to 4.0)	0.36	7.2 (2.9 to 11.5)	0.001
H6	T	A	A	A	C	C	T	C	G	3.2 (0.42 to 5.9)	0.02	2.4 (−2.4 to 7.3)	0.32
H7	C	G	A	G	C	T	C	C	G	1.1 (−2.4 to 4.5)	0.53	3.0 (−2.3 to 8.2)	0.26
H8	C	G	A	A	C	C	T	C	G	−1.7 (−19.2 to 15.8)	0.85	−2.7 (−26.2 to 31.5)	0.85
H9	C	G	A	G	C	C	C	C	G	3.4 (−6.3 to 13.1)	0.49	6.5 (−11.0 to 24.0)	0.46

AGT indicates angiotensinogen.

*Data show the *P* value after adjusting for age, sex, and body mass index.

Association Analysis Between SNPs and Plasma AGT Levels

We tested the potential association between SNPs and plasma AGT levels in 506 individuals included in the sample (386 cases and 120 controls) and identified 2 SNPs, A-20C and C3389T, associated with this trait (Table S4). The genotype association for SNP A-20C was AA=26.7±8.9 versus AC=24.1±8.2 μg/mL (P=0.003) and for AA=26.7±8.9 versus CC=22.9±8.1 μg/mL (P=0.04). For SNP C3389T, CC=26.8±8.8 versus CT=22.1±8.0 μg/mL (P=0.000001) and CC=26.8±8.8 versus TT=21.5±6.9 μg/mL (P=0.0000009). Also, the analysis with 1-way ANOVA between groups shows 2 SNPs significantly associated with plasma AGT levels, A-20C (P=0.003) and C3389T (P=0.0001). None of the alleles associated with the risk to EH (A-6, C4072, C6309, and G12775) were associated with the regulation of the plasma AGT levels.

Haplotype Association With Plasma AGT Levels and EH Risk

Nine haplotypes accounted for 93% of all potential combinations. Association between haplotypes and plasma AGT levels is shown in Table 1. In the control group, 2 haplotypes showed association with plasma AGT level: haplotype 3 (H3: CGCATCCCG) was associated with decreased plasma AGT level (−4.3 μg/mL [95% CI: −7.6 to −1.0]; P=0.01), and haplotype 5 (H5: CGCACCCCG) was significantly associated with increased plasma AGT level (7.2 μg/mL [95% CI: 2.9–11.5]; P=0.001). In the cases, H3 was also associated with decreased plasma AGT level (−3.6 μg/mL [95% CI: −6.3 to −0.9]; P=0.008), and haplotype 6 (H6: TAAACCTCG) was significantly associated with increased plasma AGT level (3.2 μg/mL [95% CI: 0.42–5.90]; P=0.02). This association is reported after full adjustment for covariates, that is, age, sex, and BMI. The total contribution of the haplotypes and covariates on plasma AGT level variance was 14%. A likelihood ratio test for haplotype-phenotype association, adjusted for covariates, that is, age, sex, and BMI, resulted in a χ^2 of 36.8 (P=0.00001). The haplotypes H1 and H5 showed association with EH with χ^2=8.1 (P=0.004) and χ^2=5.1 (P=0.02), respectively. In addition, haplotypes H2 and H4 showed protective effect with χ^2=5.1 (P=0.02) and χ^2=8.0 (P=0.004), respectively. However, only 2 remained significant after 10 000 permutations, H1 (P=0.04) and H4 (P=0.04; Table 2).

Association of Plasma AGT Levels and EH Risk

Age, sex, and 2 polymorphisms, A-20C and C3389T, were the independent variables that affected plasma AGT levels in

Table 2. Effects of Haplotypes on EH Risk

Haplotypes														
	−532	−217	−20	−6	3889	4072	6309	11535	12775	Frequency	Case/Control	χ^2	*P*	*P**
H1	C	G	A	A	C	C	C	C	G	0.39	0.42/0.32	8.1	0.004	0.04
H2	C	G	A	G	C	T	T	A	A	0.15	0.14/0.19	5.1	0.02	0.13
H3	C	G	C	A	T	C	C	C	G	0.13	0.13/0.12	0.2	0.69	1.0
H4	C	G	A	G	C	T	T	C	A	0.10	0.08/0.14	8.0	0.004	0.04
H5	C	G	C	A	C	C	C	C	G	0.07	0.08/0.04	5.1	0.02	0.12
H6	T	A	A	A	C	C	T	C	G	0.07	0.07/0.08	1.3	0.25	0.80
H7	C	G	A	G	C	T	C	C	G	0.04	0.04/0.03	0.13	0.71	1.0
H8	C	G	A	A	C	C	T	C	G	0.01	0.01/0.02	0.60	0.437	0.8
H9	C	G	A	G	C	C	C	C	G	0.01	0.01/0.01	0.01	0.911	1.0

EH indicates essential hypertension.

*Data show the *P* value after 10 000 permutations.

Table 3. Haplotype Composition and Global EH Risk

Haplotypes								
	−20	−6	3889	4072	6309	12775	Increased AGT*	Increased Risk†
H0‡	C	A	C	C	C	G	NA	NA
H1	A	A	C	C	C	G	β0	++
H5	C	A	C	C	C	G	+	+
H8	A	A	C	C	T	G	NS	NS
H6	A	A	C	C	T	G	+	NS
H3	C	A	T	C	C	G	...	NS
H7	A	G	C	T	C	G	NS	NS
H9	A	G	C	C	C	G	NS	NS
H2	A	G	C	T	T	A	NS	−
H4	A	G	C	T	T	A	NS	...

EH indicates essential hypertension; NA, not applicable; NS, not significant. Data show the + presence and − absence of alleles associated with plasma angiotensinogen or with essential hypertension risk.

*+=only in cases or controls; ++=in both cases and controls groups.

†+=$P<0.05$ without permutations; ++=$P<0.05$ after 10 000 permutations.

‡Data are based on the nucleotide present at the orthologous position in *Pan troglodytes* (NC_006468.2) for each of the human *AGT* single nucleotide polymorphisms.

the Mexican-Mestizo population. However, the plasma AGT level alone is not associated with BP-related traits after adjustment for age, sex, and BMI covariates (Table S5). Furthermore, the plasma AGT level was associated with a marginally increased EH risk (OR: 1.04 [95% CI: 1.01–1.07]; $P=0.01$), but this association was decreased after an adjustment for sex, age, BMI, and rs2493132.

Qualitative-Quantitative Traits

A summary of the haplotype effects on quantitative and qualitative traits and the haplotype SNP compositions is shown in Table 3. The H1, with a population frequency of 0.39 in our study, contains the EH risk alleles and increased plasma AGT levels. The H5, which was associated with increased plasma levels and with the EH risk, contains alleles associated with EH risk and C3389 but not the A-20 allele. The H2 and H4, with population frequencies of 0.15 and 0.10, respectively, displayed protective effects and contain alleles associated with increased plasma AGT levels and diastolic BP but do not contain EH risk alleles. The H3, which is associated with decreased plasma AGT levels, contains the EH risk alleles but not those associated with increased plasma AGT levels. Interestingly, H3 itself did not increase the EH risk although it contains hypertensive alleles; however, if the haplotype gained the hypertensive properties of C3389 (H5), the EH risk was increased, suggesting that there is a synergistic effect of alleles. To test the combined effect of associated alleles (C3389 and C6309), we used a logistic regression analysis to assess the effect of combined genotypes in the risk to EH (Tables 4 and S6). Allele risks showed an additive effect on the development of EH. This findings support the notion of a combined effect of individual alleles in the risk for EH.

Haplotype Phylogenetic Analysis

The unrooted haplotype phylogenetic tree constructed using the neighbor-joining method is shown in the Figure. The haplotype in primates (H0) was considered as a proxy for the ancestral haplotype. Using Kimura's method[21] to infer the distance between haplotypes, we noted that the risk haplotypes H5 and H1 have a shorter distance from H0 (H0 to H5=0.12; H0 to H1=0.27) and may be considered as human ancestral haplotypes. H2 and H4 are more recent haplotypes in the evolutionary history of the *AGT* gene because they show the longest distances from H0 (H0 to H2=1.68 and H0 to H4=1.14). Figure S3 shows the phylogenetic tree using the Amerindian population (Zapotecs). We observed a decreased in haplotype diversity in this population; however, the risk haplotypes H5 and H1 were also considered as human ancestral in this population, with similar distances as the haplotypes from the Mestizo population (H0 to H5=0.13 and H0 to H1=0.31).

Discussion

The *AGT* gene is the only one for which association with EH has been consistently replicated in multiple populations.[13] Several polymorphisms across the *AGT* gene have been associated with both EH and plasma AGT levels. Increased plasma AGT levels have been postulated as a causal factor for increased risk to EH. However, the lack of replication across populations has prevented the translation of this association

Table 4. Logistic Regression Analysis for Combined Associated Alleles

SNPs	OR	SE	*Z*	*P*	95% CI
C3889T	0.93	0.26	−0.25	0.80	0.53–1.62
C6309T	1.81	0.34	3.14	0.002	1.25–2.62
AGT1	1.30	0.64	0.53	0.59	0.49–3.45
AGT2	2.13	0.76	2.13	0.033	1.06–4.30
AGT3	3.33	1.4	2.82	0.005	1.44–7.69

OR indicates odds ratio; SNPs, single nucleotide polymorphisms. AGT1 indicates the combination of 1 and 2 risk alleles; AGT2, combination of 3 risk alleles; AGT3, combination of 4 risk alleles.

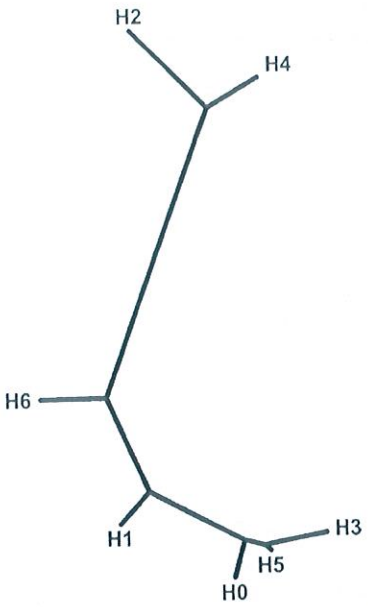

Figure. Unrooted phylogenetic tree constructed by the neighbor-joining method with 6 common Mexican Mestizo *AGT* haplotypes. The network includes a hypothetical ancestral *AGT* haplotype (H0), which is based on the nucleotide present at the orthologous position in *Pan troglodytes* (NC_006468.2) for each of the 9 human *AGT* single nucleotide polymorphisms.

to clinical applications. Differences in the genomic structure between human populations, including that of the Mexican population,[18] have been described in the last decade, and these differences might be the source for this lack of replication. Thus, analysis of the risk-associated alleles in multiple interethnic populations may become an important tool to determine the role of genetic predisposition in the development of the disease.

This study identified 4 alleles associated with EH risk and were found to be independent risk factors in a logistic regression model. We were able to replicate the 2 most important associations, A-6 and C4072 (T235), and we identified 2 new markers associated with higher risk, C6309 and G12775. The association signal at intron 2 of the *AGT* (C6309) is particularly interesting because another polymorphism was found recently in intron 2 (C6233T) associated with a major effect on the risk to EH in whites.[8] An important point in our study is that we did not find any association between these SNPs and plasma AGT levels in the Mexican population, which reproduces the negative association between C4072T (T235M) and plasma AGT reported previously in a Mexican-derived population.[6,23] This finding suggests that mechanisms other than an increased level of plasma AGT contribute to EH risk in the Mexican-Mestizo population. Interethnic variability in the genetic control of plasma AGT levels has been reported for Japanese and African-American children. Sato et al[14] found no association between C4072T (T235M) and plasma AGT levels, and the SNP G-1074T associated with AGT levels did not increase the EH risk. In another study, Bloem et al[24] were also unable to find an association between plasma AGT levels and C4072T (T235M).

In the Mexican-Mestizo population, the A-20C and C3389T (T174M) were found to be associated with plasma AGT levels, and the C3389 (T174) allele showed the strongest association. However, these SNPs were not associated with increased risk for EH. Both SNPs have been associated with EH and plasma AGT levels in other populations. For example, A-20C located in the promoter region of *AGT* is associated with both in vitro changes in *AGT* transcription and plasma AGT levels in the Japanese population.[25,26] In a meta-analysis including 11079 subjects, an association between C3389T (T174M) and EH was identified in Asian and multiethnic populations but not in a European population.[15] The functional mechanism by which C3389T (T174M) increases BP among carriers is currently unknown, and several studies have failed to show association between this SNP and plasma AGT levels.[3,27,28] In our sample, levels of plasma AGT by itself did not increase the risk of EH nor showed association with any BP-related traits in our covariate-controlled linear regression analysis. In addition, a recent study failed to associate plasma AGT levels and BP-related traits in a family cohort of white ancestry.[29] These results differ from those in an initial report that found a positive correlation between plasma AGT levels and diastolic BP.[30] The observations that plasma AGT levels are not associated with any BP-related traits and are not independent risk factors for EH support the notion that, at least in our sample, the increased EH risk from the *AGT* locus involves mechanisms other than an increase in plasma AGT levels alone.

To summarize these results, individual SNP analysis identified polymorphisms that were associated with EH risk but not were associated with plasma AGT levels; we also identified SNPs associated with plasma AGT levels that were not associated with EH risk. Plasma AGT is not an independent risk factor for EH and does not impact the BP-related trait.

If we consider that each SNP in a genomic region is fixed to another SNP by evolutionary forces, it would be anticipated that this haplotype background could be more informative than individual SNPs alone. Several publications have described the higher informative value of haplotype analysis as compared with individual SNP analysis.[8,31–33] Our haplotype analysis found that the H1 and H5 are associated with risk to EH, and the H2 and H4 are associated with protection against EH. The lack of association between haplotypes H2 and H5 with hypertension after a permutation test could be influenced by sample size and modest haplotype frequency differences between cases and controls. For this reason, a replication study with a larger sample size could be useful to strengthen this analysis and contribute to clarifying the effect of these haplotypes on the risk of EH.

The SNP composition of these haplotypes shows that H1 contains the EH risk alleles and plasma AGT levels. The second risk-associated haplotype H5 also contains the EH risk alleles but lacks the A-20 allele in the promoter, similar to one of the ancestral haplotypes. Interestingly, H3, which includes the EH risk alleles but lacks the plasma AGT levels, did not increase the risk for EH. The protective haplotypes, H2 and H4, include plasma AGT level–associated alleles but not those from the EH risk alleles. Our analysis suggests a more complex model than a single polymorphism effect that

involves a combination of variants within the *AGT* gene, which modulate the risk for EH and plasma AGT levels. There are multiple indications that several polymorphisms within a gene position interact to affect quantitative trait variation. Thus, multiple locus interactions create a major locus that has a large effect on the observed phenotype (superallele).[34] Quantitative trait mapping in *Drosophila melanogaster* has shown that major gene effects are not necessarily attributed to single site polymorphisms but are the result of the combined effects of multiple associated polymorphisms.[35] This phenomenon has also been described in human traits and diseases; for example, the apolipoprotein gene (*APOB*) affects plasma low-density lipoprotein and high-density lipoprotein cholesterol,[36] the *LCT* gene influences intestinal lactase activity,[37] and ADRB2 influences the actions of catecholamines on bronchodilation and risk to asthma.[38] Similarly, the *AGT* gene has a demonstrated additive effect on the risk to EH, acting as a superallele, when specific SNPs are present.[39,40]

Genetic distance analysis on these haplotypes showed that H5 and H1 have the shortest distances from the ancestral chimpanzee haplotype. Considering that haplotypes H1 and H5 contain the major alleles and have the shortest distances with respect to the ancestral haplotype H0, we named these as human ancestral. The difference between H1 and H5 haplotypes is the presence of the A-20 allele in H5 and H0. The H2 and H4 have the longest distances from H0, suggesting a recent expansion of haplotypes. The Zapotecs, an ancestral Amerindian population, contain a similar genetic distance pattern for H1 and H5, supporting the ancestral character of these haplotypes and also the important genetic contribution of the Amerindian population on the modern Mexican-Mestizo population. These observations are in agreement with the ancestral-susceptibility model for common diseases in which the ancestral alleles reflect risk in the modern lifestyle, whereas in human ancestors these same ancestral alleles provided adaptive advantages to both a low-salt intake and vegetable-based diets.[41]

In conclusion, our results show heterogeneity in the effects of *AGT* polymorphisms on EH risk and plasma AGT levels. Two haplotypes act as superalleles for the risk to EH, containing EH risk alleles and plasma AGT levels. Two SNPs were associated with plasma AGT levels, but no association was identified between plasma AGT variation and risk to EH and BP-related traits. These findings suggest that population genetic diversity plays an important role in the control of intermediate traits and helps to elucidate interethnic variability in plasma AGT levels and the role of the *AGT* locus in EH in the Mexican-Mestizo population.

Perspectives

Our results contribute to understanding the influence of the AGT locus to the risk to EH in a population with a unique genomic ancestry as the Mexican ancestry. Expression analysis of intron 2 and its interactions with functional SNPs across the AGT may be interesting to determine its role in disease. Furthermore, cohort follow-up of genotyped individuals in the general population would be valuable to understand the effect of haplotypes on BP continuous traits and also the role of haplotypes in the clinical setting.

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Disclosures

None.

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Resequencing, haplotype construction and identification of novel variants of *CYP2D6* in Mexican Mestizos

Aim: The CYP2D6 enzyme participates in the metabolism of commonly prescribed drugs: antidepressants, antipsychotics and antihypertensives. The *CYP2D6* gene shows a high degree of interindividual and interethnic variability that influences its expression and function. Mexican Mestizos are a recently admixed population resulting from the combination of Amerindian, European and, to a lesser extent, African populations. This study aimed to comprehensively characterize the *CYP2D6* gene in Mexican Mestizos. **Materials & methods:** We performed linkage disequilibrium and network analyses in resequencing data of 96 individuals from two regions within Mexico with a different history of admixture and particular population dynamics, the Northwestern state of Sonora and the Central-Pacific state of Guerrero. **Results & conclusion:** We identified 64 polymorphisms, including 14 novel variants: 13 SNPs and a *CYP2D7* exon 2 conversion, that was assigned *CYP2D6*82* by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee. Three novel SNPs were predicted to have functional effects. For *CYP2D6*82* we hypothesize an Amerindian origin that is supported by its identification in three Mexican Amerindian groups (Mayas, Tepehuanos and Mixtecos). Frequencies of *CYP2D6*1, *2, *4, *5, *10, *29, *53, *82* and its duplications were 50.0, 25.5, 14.1, 2.0, 2.6, 1.0, 0.5, 2.1 and 3.6%, respectively. We found significant frequency differences in *CYP2D6*1* and **2* between Mexican Mestizos and in *CYP2D6*1, *2, *4, *5, *10* and **29* between Mexicans and at least one other population. We observed strong linkage disequilibrium and phylogenetic relationships between haplotypes. To our knowledge, this study is the first comprehensive resequencing analysis of *CYP2D6* in Mexicans or any other Latin American population, providing information about genetic diversity relevant in the development of pharmacogenomics in this region.

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Genetic variants in drug-metabolizing enzymes play a critical role in interindividual differences to drug response and adverse reactions [1]. The cytochrome P450 (CYP) superfamily of microsomal drug-metabolizing enzymes catalyzes Phase I drug metabolism [2], a member of this family, CYP2D6 is central to the metabolism of a large number of commonly prescribed drugs, including antidepressants, antipsychotics, antihypertensives, β-adrenergic blocking agents and antiarrhythmics. CYP2D6 shows a very high degree of interindividual variability primarily due to genetic polymorphisms that influence expression and function [3]. This variability leads to distinct functional phenotypes termed ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM) [4,5]. Whereas poor metabolism contributes to dose-related adverse events, UM patients fail to respond to conventional doses of drugs metabolized by CYP2D6 [3,4].

The *CYP2D6* gene is part of the *CYP2D* cluster on chromosome 22 together with two pseudogenes, *CYP2D7* and *CYP2D8* [6]. *CYP2D6* is highly polymorphic having more than 75 alleles described to date [101]. The frequencies of *CYP2D6* functional and nonfunctional alleles vary among ethnic groups, both within and between populations. In populations of European descent the predominant variants are functional *CYP2D6*1* and *CYP2D6*2* alleles, which together have a frequency of approximately 70%, followed by the nonfunctional *CYP2D6*4* allele with a frequency range of 17.5–23% [7,8]. In Asians, Africans and African–Americans, functional alleles have a frequency of approximately 50%, and *CYP2D6*4* of approximately 5% [8,9]. In Amerindian populations, functional alleles have a frequency ranging from 64% in Native Americans from Argentina and Paraguay [10] to 99% in Tepehuanos from Mexico [11], in these populations, the highest frequency for the nonfunctional *CYP2D6*4* allele was approximately 15% in natives from Panama and Colombia [12] and the lowest was 0.6% in Tepehuanos from Mexico [11]. The *CYP2D6*10* gene, which produces a low-activity enzyme, is

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Table 1. Novel polymorphisms of *CYP2D6* identified in the Mexican population.

Position[†]	Location	Flanking sequence 5′–3′ direction	Nucleotide change[‡]	Predicted effect	SIFT/ PolyPhen[§]	HWPval	MAF (%)			p-value[¶]
							GUE (n = 50)	*SON (n = 46)*	*MEX (n = 96)*	
729	P	GGGCTCTGGCg/tGGGCTATGGC	G>T	-	-	1.0	1.0	0.0	0.5	1.404
2633	**E2**	**GGTGATGGGCt/cCAGGCGGGCG**	**T>C**	**Val104Ala**	**-/-**	**1.0**	4.0	0.0	2.1	0.140
2641+ 2642	**E2**	**CAGGATCTGGac/taGATGGGCACA**	**AC>TA**	**Thr107Tyr**	**-/-**	**1.0**	4.0	0.0	2.1	0.140
2647	**E2**	**AAACCCAGGAa/gCTGGGTGATG**	**A>G**	**Ile109Val**	**-/-**	**1.0**	4.0	0.0	2.1	0.140
2686	I2	CTCTGTCCCCt/gCCGCTGCTTG	T>G	-	-	1.0	0.0	1.1	0.5	0.883
2769	I2	CCTGTTTCATc/gTCCACGACCC	C>G	-	-	1.0	0.0	1.1	0.5	0.883
2798	I2	TTTCTTGGCCg/cGCTGTCCCCA	G>C	-	-	1.0	0.0	1.1	0.5	0.883
2914	I2	CTGGGAAATGg/aGCCACGCTCA	G>A	-	-	1.0	0.0	1.1	0.5	0.883
2975	I2	ACCCACACTGt/cGCTTACAGCA	T>C	-	-	0.005	0.0	2.2	1.0	0.353
3229	E3	GCGCCAGGAAg/aACCCCTGGGG	G>A	Val119Val	NA	1.0	0.0	1.1	0.5	0.907
3742	I4	GGGGTCTCCTc/tGAATGTCCTT	C>T	-	-	1.0	2.0	0.0	1.1	0.647
3834	I4	CCTTGGCCTCa/gCCTGGTGCCC	A>G	-	-	1.0	2.0	0.0	1.1	0.654
3911	I4	CCAGCCTCCAg/aCCTCTCTCCT	G>A	-	-	1.0	2.0	0.0	1.1	0.630
4779	E7	CTGTTGGACAg/cGGCCTGGACA	G>C	Arg330Pro	++/++	1.0	0.0	1.1	0.5	0.883
5647	I8	CTGTGGGGAGc/aGGAGGGGCGT	C>A	-	-	1.0	0.0	1.1	0.5	0.883
5688	E9	CTCCATGCGGc/aCCAGGGGCTC	C>A	Ala449Asp	++/++	1.0	4.0	3.3	3.6	1.001

Bold sites are part from a novel multibase allelic CYP2D7 exon 2 conversion.
[†]Position in the gene from accession number M33388.
[‡]Nucleotide change considering NCBI sequence database.
[§]Coding for potential functional effect (used software): ++: probably damaging (PolyPhen) or deleterious (SIFT); +: possibly damaging (PolyPhen) or deleterious with low confidence (SIFT); -: benign or tolerated.
[¶]Comparison of the MAF between two Mexican Mestizo subpopulations (p-value).
E: Exon; GUE: Guerrero; HWPval: Hardy–Weinberg equilibrium (p-value); I: Intron; MAF: Minor allele frequency; MEX: Mexico (SON + GUE); NA: Not applicable; P: Promoter; SON: Sonora.

the predominant variant in Asians with a frequency as high as 52% in the Chinese [13] and 45% in Koreans [14]. In other populations the *CYP2D6*10* is a low-frequency allele, having a frequency of approximately 1–8% in populations of European origin [15,16], and of 17, 7 and 2% in the Ngawbe, Embera and Mapuche Amerindian groups, respectively [17,18]. In other Amerindian groups, such as Pima, Maya and Colombian, this allele has not been found [19].

There are few studies on polymorphisms of *CYP2D6* in the Mexican population or other admixed Latin American populations, and most of them only included the analysis of alleles described by the P450 Nomenclature Committee [11,20–24]. The Mexican population is mainly composed by Mestizos with a mixed genetic background of Amerindian, European and, to a lesser extent, African ancestries [25,26]. According to the Mexican Genome Diversity Project (MGDP), genetic differences in Mexican Mestizos from distant geographically regions is due to different population dynamics, related to both the intermixture proportions of different ancestral components and demographic conditions [27,28]. Results of this project showed that Mexicans from Sonora had the highest European (0.616 ± 0.085) and the lowest Amerindian (0.362 ± 0.089) ancestral contributions; whereas individuals from Guerrero had the highest Amerindian (0.660 ± 0.138) and the lowest European (0.285 ± 0.120) ancestral contributions [28]. We believe that targeted resequencing of biologically relevant genes in admixed populations with known differences in ancestral contributions and of Amerindian groups is a useful approach to identify new variants that could be analyzed further to help understand relationships between genotypes and phenotypic conditions in population groups with similar ethnic origin. To the best of our knowledge, this is the first effort to sequence the entire *CYP2D6* gene in Mexicans, including 1.6 kb of the 5′ flanking sequence. We included Mexican Mestizos from the Northern state of Sonora (SON) and from the Center-Coastal state of Guerrero (GUE), both samples are part of the MGDP [28]. Our results describe *CYP2D6* genetic diversity in Mestizo subpopulations with known differences in ancestral contributions and contribute to the identification of novel genetic variants and haplotypes in one of the most important genes related to drug response. These results may help clarify the relationship between ethnicity and phenotype to improve implementation of pharmacogenomics to clinical practice.

Materials & methods

Subjects

We resequenced *CYP2D6* in 96 Mexican Mestizos, 50 from the state of GUE and 46 from the state of SON, all of them recruited in the MGDP, a comprehensive description of this process has been described [28]. Genomic DNA was extracted from buffy coats isolated from blood samples using QIAamp® DNA Blood Maxi Kit (QIAGEN GmbH, Hilden, Germany). Validation of novel variants was carried out by targeted resequencing of 120 Mexican Mestizos (n = 30 from each of four different states: Guanajuato, Veracruz, Yucatán and Zacatecas); as well as 120 Amerindians (n = 30 from each of four groups: Tepehuanos from Durango, Mayas from Campeche, and Mixtecos and Zapotecos from Oaxaca; SUPPLEMENTARY FIGURE 1; www.futuremedicine.com/doi/suppl/10.2217/pgs.11.8). Self-identified ethnicity of all participants was registered, as well as for their parents and four grandparents. All participants signed the informed consent in their language. This study is part of the extensive genotypic characterization foreseen in the MGDP that was approved by the Scientific, Ethics, and Biosafety Review Boards from the National Institute of Genomic Medicine (INMEGEN).

CYP2D6 resequencing

To resequence 1.6 kb of 5′-flanking sequence, all nine exons and eight introns of *CYP2D6*, we amplified four overlapping fragments – promoter (1669bp), A (2589 bp), B (1830 bp) and C (1316 bp) – on a GeneAmp® PCR System 9700 (Applied Biosystems, CA, USA) according to previously described protocols, using the Expand High Fidelity PCR System (Roche Diagnostics GmbH, Mannheim, Germany) [29,30]. These fragments were used as templates in independent PCRs to obtain shorter overlapping subfragments, with the exception of fragment B that was successfully sequenced using nested primers. These amplifications (584 bp on average) were performed with a proofreading DNA polymerase (Platinum® Pfx DNA Polymerase, Invitrogen, CA, USA) in a total volume of 50 µl PCR. Final concentrations were the following, dNTP mixture (Takara Bio, Inc., Shiga, Japan) 0.3 mM, $MgSO_4$ 1 mM, Primer mix (10 µM each) 0.3 mM, Platinum Pfx DNA Polymerase 1.5 U and DNA template 100 ng. The PCR products were cleaned-up using QIAquick® PCR Purification Kit (Qiagen GmbH), and then sequenced using the BigDye® Terminator

Table 1. *CYP2D6* allele frequencies in two Mexican Mestizo subpopulations and among Mexican population studies.

CYP2D6 alleles	This study			Sosa-Macias *et al.* (n = 110) [11]		Lopez *et al.* (n = 243) [20]		Mendoza *et al.* (n = 349) [22]		Luo *et al.* (n = 264) [21]	
	GUE (n = 50) Freq. (%)	*SON (n = 46) Freq. (%)*	*MEX (n = 96) Freq. (%)*	*Freq.(%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]
*1	60.0	39.1	50.0			19.34	0.055	22.8	0.241	55.1	0.129
*2	17.0	34.8	25.5			1.44	0.096	0.3	0.615	18.0	0.018
*3	0.0	0.0	0.0	0.9	0.285	11.21	0.195	10.3	0.093	0.2	0.733
*4	14.0	14.1	14.0	13.1	0.453	2.67	0.447	2.3	0.560	10.1	0.085
*5	2.0	2.1	2.0			12.45	<0.0001	7.4	0.008	1.7	0.472
*10	2.0	3.3	2.6	2.3	0.538	1.65	0.069	0.7	0.296	2.8	0.549
*17	0.0	0.0	0.0							0.2	0.733
*29	2.0	0.0	1.0							0.2	0.175
*53	0.0	1.1	0.5								
*82	4.0	0.0	2.1			12.76	<0.0001	1.0	0.017	0.8	0.010
Duplication	3.0	4.3	3.6								
Novel haplotypes[‡]	1.0	3.2	2.1								

[†]*p-value is for the comparison of the allele frequencies for Mexicans between our results (including GUE and SON) and data reported by other authors.*
[‡]*Novel haplotypes carrying only 1661G>C or 4180G>C.*
Freq.: Frequency; GUE: Guerrero; MEX: Mexico (SON + GUE); SON: Sonora.
Data taken from [11,20–22].

V3.1 on a 3730xl DNA Analyzer (Applied Biosystems). Results were analyzed using Module SeqMan V7.0 (Lasergene software V7.0, DNASTAR, Inc.). The reference gene sequences were obtained from GenBank-accession numbers: M33388 [6], AY545216 [31] and NG_008376.1 (Genome Annotation REFSEQ, build 36 version 3 of NCBI) [32].

CYP2D6 copy number assay

To genotype *CYP2D6* copy number variants we used a TaqMan® Gene Copy Number Assay (Applied Biosystems) with two primers and a FAM-probe in 20× formulation (Hs00010001_cn). We used 25 ng of genomic DNA as template and we ran it as a TaqMan real-time PCR reaction with the RNase P gene (VIC/Tamra probe) as internal standard in the same well (two copies present in a subject with or without duplication/deletion of *CYP2D6*). *CYP2D6* copy number was determined by the comparative Ct method [33] running three replicates for each DNA sample on the same plate. We used the relative quantification assay; therefore, a reference sample (calibrator) from the Coriell Institute for Medical Research (NA17122) having two copy numbers for *CYP2D6* assay was chosen.

Allele frequencies

Allele frequencies were calculated by the counting method and were tested for Hardy–Weinberg equilibrium with a Fisher exact test implemented in De Finetti generator v.3.0.5 [102]. Comparison of allele frequencies between the Mexicans and other populations was carried out using a Fisher exact test (SPSS Statistics version 13). The level of statistical significance was defined as $p < 0.05$.

Haplotypes inference

Haplotypes were inferred from individual genotypes using the software PHASEv2.1 [34,35]. Conditions employed for the haplotype inference procedure using 64 biallelic SNPs consisted in 500 iterations and five thinning interval steps through the Markov chain.

Pairwise genetic distance

Fst index-based genetic distance was calculated for GUE and SON Mestizo subpopulations using Arlequin v3.11 [36].

Identification of potential functional novel variants

SIFT [37]!boe!QpmzQifo![38]!xfsf!vtfe!up!qsfejdu!uif!fggfdu!pg!opotzopozn pvt!dpejoh!TOQt!po!DZQ3E7!qspufjo!gvodujpo/

Mjol bhf!ejtfrvjmjcsjvn !' !ofux psl !pg!i bqmpuzqft

Mjol bhf!ejtfrvjmjcsjvn !)ME*!boe!i bqmpuzqf!cmpdl !bobmztft!xfsf!qfsgpsn fe!xjui !I bqmpwjfx!5/1![39,103]; and tagSNP identification was carried pvu!xjui !Ubhhfsljn qmfn fouf e!jo!ui jt!tpgux bsf/!

Ubcmf!4/!DZQ3E7!hfopuzqft!jo!uxp!Nfyjdbo!Nftuj{p!tvcqpqvmbujpot/

DZQ3E7!hfopuzqft	Gsfrvfodz!)&*		
	HVF!)o!>!61*	TPO!)o!>!57*	NFY!bwh/!)o!>!:7*
+20+2	45/1	28/5	36/8
+20+3	33/1	32/8	32/:
+20+5	25/1	24/1	24/6
+20+21	3/1	3/3	3/2
+20opwfmi bqmpuzqf[a]	3/1	1/1	2/1
+20+93	7/1	1/1	4/1
+20+64	1/1	3/3	2/2
+30+3	3/1	2: /7	21/9
+30+5	7/1	5/4	6/3
+30+21	1/1	3/3	2/2
+30+93	3/1	1/1	2/1
+50+5	1/1	3/3	2/2
+50+21	3/1	1/1	2/1
+50+3:	3/1	1/1	2/1
+60+2	3/1	3/3	3/2
+60+5	3/1	3/3	3/2
Opwfmi bqmpuzqf0opwfmi bqmpuzqf[a]	1/1	3/3	2/2

[a]Opwfmi bqmpuzqf!dbsszjoh!TOQ!obnfe!2772H?D!ps!5291H?D/
bwh/;!Bwfsbhf<!HVF;!Hvfssfsp<!NFY;!Nfyjdp<!TPO;!Tpopsb/

Phylogenetic relationships of haplotypes were built with Network 4.516 [104], a software based on the median-joining algorithm [40].

Transcriptional factor-binding sites

Putative transcriptional factor-binding sites were identified with the MatInspector module of Genomatix software [105].

Results

In our Mexican sample, we observed 64 genetic polymorphic sites in *CYP2D6* (Supplementary Table 1), including 13 novel SNPs and a novel *CYP2D7* exon 2 conversion, that was assigned the *CYP2D6*82* allele designation by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (Table 1). All positions were determined using three different reference sequences and including 20 bases of flanking sequence, known variants were named according to the Human Cytochrome P450 Allele Nomenclature Committee indicated by their respective identification notation. Ten SNPs were detected in the 5′-flanking region, including a novel one. In total, 21 SNPs were found in the coding region, including three novel ones: a synonymous SNP in exon 3 (Val119Val), and two nonsynonymous SNPs, one in exon 7 (Arg330Pro) and the second in exon 9 (Ala449Asp). All novel SNPs in the coding region will be published in the SNP table on the CYP allele website [101]. The novel non-synonymous SNPs were predicted to be damaging by PolyPhen and SIFT (Table 1). We observed 23 intronic polymorphisms, including the known *CYP2D7* intron 1 conversion; 13 previously described SNPs, and nine novel SNPs. The comparison between minor allele frequencies (MAF) of the two Mestizo subpopulations analyzed showed that ten polymorphisms were significantly different ($p \leq 0.05$), including the known *CYP2D7* intron 1 conversion (Supplementary Table 1).

Characterization of *CYP2D6* alleles & genotypes in Mexicans

CYP2D6 alleles were identified with all polymorphisms found by direct sequencing. *CYP2D6* haplotypes were inferred from genotypes of 96 individuals from GUE and SON (Table 2). The most frequent allele was wild-type *CYP2D6*1* (50.0%), of which 3.12% corresponds to a novel variant of this allele including rs1081004 (named by us as *CYP2D6*1V*). The second most frequent was *CYP2D6*2* (25.5%), of which 18.6% corresponds to three novel suballeles (named by us as *CYP2D*2V*, **2W* and **2R*) (Supplementary Table 2). *CYP2D6*4*, harboring a splicing defect (1846G>A), frequency was 14.0%, for it two suballeles were identified, one includes a novel SNP (Ala449Asp) and was named by us as **4W*. The frequencies of the entire gene deletion (*CYP2D6*5*), and of the gene duplication were 2.0 and 3.6%, respectively. We observed gene duplications in seven individuals with three *CYP2D6* copies, in five of them we identified only functional alleles and in two we identified one functional and one nonfunctional allele, however, we are not certain of the specific allele being duplicated owing to the limitations of our assay. The less frequent alleles found were *CYP2D6*29* (1.0%) and *CYP2D6*53* (0.5%). In addition to known alleles, we identified two novel *CYP2D6* haplotypes bearing one of the following known SNPs: 1661G>C (**1661*) or 4180G>C (**4180*). Together these novel haplotypes had a frequency of 3.2% in SON and 1.0% in GUE (Table 2). Statistically significant differences were only observed between SON and GUE for the *CYP2D6*1* and *CYP2D6*2* alleles ($p \leq 0.005$). To measure the genetic distance due to genotypic variability in *CYP2D6* between the analyzed Mestizo subpopulations (GUE and SON), we performed a pairwise *Fst* analysis and found an *Fst* = 0.05 ($p < 0.00001$). In the genotype analysis, we identified 20 genotypes for *CYP2D6* (Table 3). Genotypes including two functional alleles comprise 58.4%, and genotypes including nonfunctional *CYP2D6*4* alleles had frequencies of 20.7% for heterozygotes, 1.1% for homozygotes and 2.1% for the *CYP2D6*41/CYP2D6*5* combination.

LD & tag SNPs

Linkage disequilibrium was evaluated using SNPs with a MAF ≥ 0.05 (Figure 1). Pairwise LD analysis of SNPs across the 5.7 kb of *CYP2D6* showed strong LD spanning the, entire region, with most SNPs pairs with a D' value equal or close to 1.0. Such significant LD in our population is consistent with that observed in other non-African populations [19,41,42] suggesting low recombination within the *CYP2D6* locus in Mexicans. We selected Tag SNPs in *CYP2D6* that capture information on other variants with MAF ≥ 5% and we identified six potentially useful in association studies in Mexicans (Supplementary Figure 2).

Identification of a novel *CYP2D7* gene conversion in Mexicans (*CYP2D6*82*)

We found a fragment of *CYP2D7* sequence in exon 2 in a *CYP2D6*1* background in four individuals from GUE (4%). This sequence

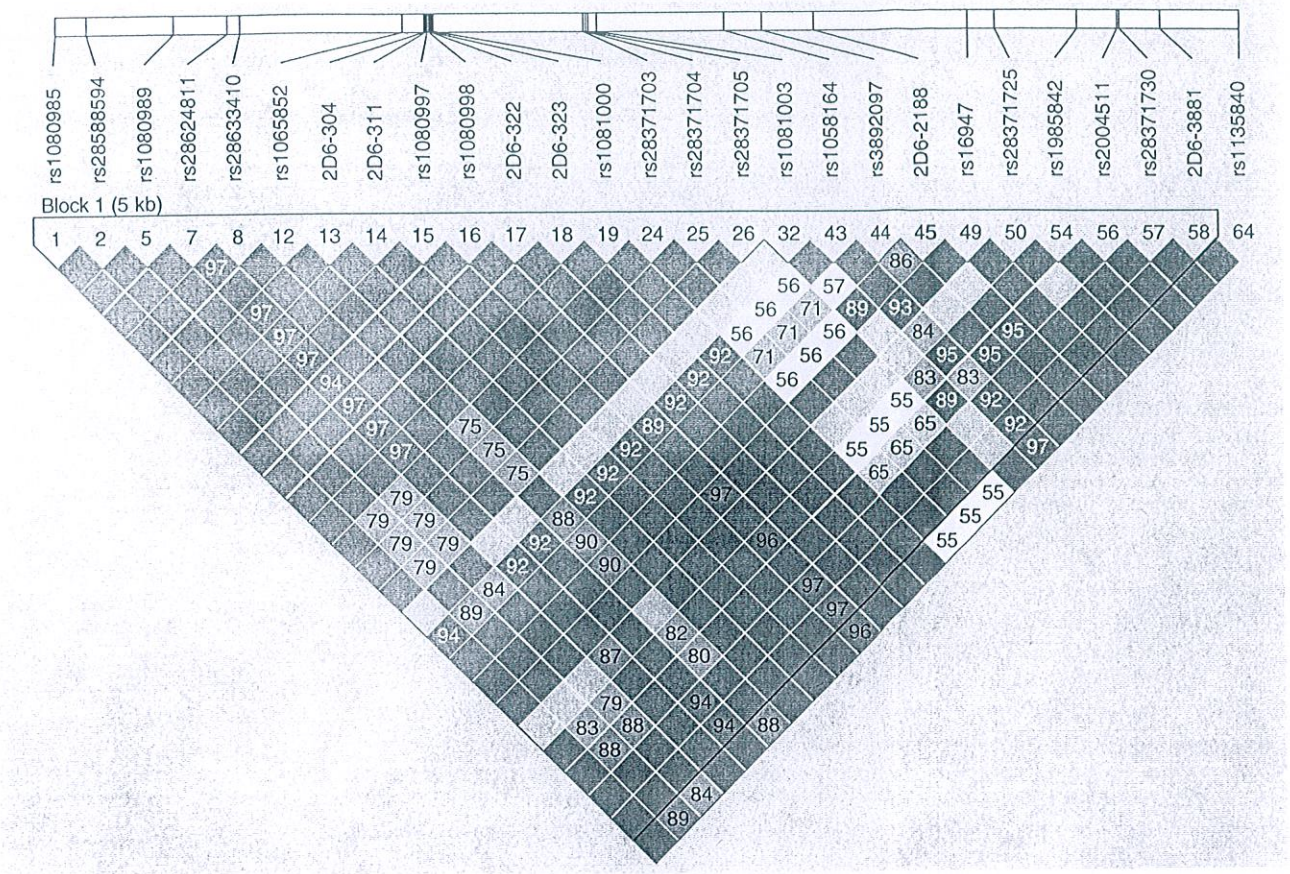

Figure 1. Linkage disequilibrium analysis of *CYP2D6* polymorphisms. Linkage disequilibrium (LD) is displayed in dark gray for LOD ≥ 2 and D′ = 1, and shades of gray for LOD ≥ 2 and D′ < 1, LOD < 2 and D′ = 1 and LOD < 2 and D′ < 1. SNPs with a minor allele frequencies ≥ 0.05, were named using its dbSNP ID (rs); for those SNPs without dbSNP ID the position from NCBI Build 36 with 2D6-.

comprises at least 62 bp from 974C>A to 1036T>C, according to the Human Cytochrome P450 Allele Nomenclature Committee [106], or from 1063C>A to 1125T>C, according to NCBI Build 36 reference sequence NG_008376.1 RefSeqGene. The *CYP2D6*82* allele includes four known SNPs, 974C>A (Leu91Met), 984A>G (His94Arg), 997C>G (Thr98Thr) [43] and rs1135821 (Gly111Gly); and four novel ones 1014T>C (Val104Ala), 1022A>T+1023C>A (Thr107Tyr) and 1028A>G (Ile109Val). The fact that GUE is a Mestizo subpopulation with high Amerindian ancestry [28], suggests an Amerindian origin for this novel allele. To support this we resequenced this region in four Mexican Amerindian populations (Mixtecos, Tepehuanos, Mayas and Zapotecos); and four Mestizo groups (YUC, ZAC, DGO and VER). We identified *CYP2D6*82* in three of four Amerindian groups, Mixtecos (5%), Tepehuanos (3.3%) and Mayas (1.67%), and also in two of four Mestizo subpopulations, YUC (3.4%) and ZAC (1.67%) (Table 4).

Network analysis of *CYP2D6* haplotypes

We used all the identified *CYP2D6* haplotypes with a frequency of ≥1% to do the network analysis. All new haplotypes were labeled with a capital letter (R, S, V, W, Z) (Figure 2). This analysis suggested that 15 haplotypes have phylogenetic relationships that separated them in four branches. The functional *CYP2D6*1A* and *CYP2D6*2* alleles were the most frequent variants in Mexican Mestizos. The network also showed that derived variants leading to null or impaired metabolic activity such as **4* and **10* are equally distributed in different geographic regions in Mexico. The novel haplotype corresponding to the *CYP2D6*82* allele was restricted to GUE.

Discussion

CYP2D6 is a highly polymorphic gene involved in the biotransformation of numerous clinically used drugs. We estimate that we have detected over 99% of polymorphic sites with a frequency of at least 1% in Mexicans, in the

192 haploid genomes included in our study, and approximately 95% of all polymorphic sites with a similar frequency in each of the subpopulations included (GUE and SON) [44]. Among the *CYP2D6* variants identified the most common were the functional *CYP2D6*1* and *CYP2D6*2* alleles and the nonfunctional *CYP2D6*4* allele, followed by reduced-function *CYP2D6*10* and *CYP2D6*29* alleles (TABLE 2). Studies in African–Americans or South African populations have shown a tendency toward slower CYP2D6 activity, attributed in part to the presence of the *CYP2D6*17* allele with a frequency of 19.1% [45] and 12.6% [46], respectively. However, in Latin American populations this allele has a low frequency, 1.7% in Colombia [23] and 0% in Nicaraguan Mestizos [47], and the Mexican Mestizos in our study. By contrast, we observed *CYP2D6*29* [19] in our GUE sample (2%), an allele present in Central/South Asian populations. These findings are consistent with the fact that Mexican Mestizos have a larger Asian contribution mainly represented in their Amerindian ancestral origin, compared with the low African ancestral contribution, as has been previously shown [28]. *CYP2D6*53*, a low frequency (0.5%) allele in our population, is a rare variant in Japanese (0.2%) [48] and it is known to have a higher activity than *CYP2D6*1* [49]. To our knowledge this is the first study that identifies *CYP2D6*53* in Mexicans, in which its origin could be related to the Amerindian ancestry.

In the copy number variant (CNV) analysis, the observed frequency of the gene deletion and the duplication were 2.0 and 3.6%, respectively. We identified *CYP2D6* CNVs with an assay targeting a specific site of exon 9 that accurately quantifies all non-*CYP2D6*36* alleles [50]. We believe this assay is adequate for our study because *CYP2D6*36* has only been reported in African–Americans and Asians [51] and we did not find it in Mexicans; however, through our analysis we cannot discard its potential presence in this population. Assays targeting other regions of *CYP2D6* (e.g., 5′-flanking region), could be used in the future to detect *CYP2D6* duplication arrangements or certain chimeric genes in Mexicans [52,53].

We identified intrapopulation differences in allele frequencies ($p \leq 0.005$) among Mexican Mestizos from GUE and SON in *CYP2D6*1* and *CYP2D6*2*. These differences are possibly due to the known higher European and lower Amerindian contributions in the SON mestizos compared with the GUE sample [28]. This observation is supported by the fact that *CYP2D6*1* and *CYP2D6*2* were significantly different between Spaniards and GUE and not between Spaniards and SON [54]. Interestingly, the genetic distance estimation between SON and GUE using *CYP2D6* haplotypes ($Fst = 0.05$) is higher than that reported using genome-wide data ($Fst = 0.019$) [28], this could be related to the highly polymorphic nature of this genetic region, or to an underestimation of genetic variation in Mexican Mestizos when a genome-wide genotyping platform is used [28]. Genome-wide SNP genotyping platforms suffer from ascertainment bias because their design is based in common variants in European and Asian populations [55]. A clearer picture of this statistic will be obtained in the future when similar analyses are performed with genome-wide resequencing data. This result supports the known population structure in Mexican Mestizos due to differences in ancestral contributions [28], and underscores the relevance of the nature and number of genetic markers used in genetic difference estimations between human groups.

Although the observed average allelic frequencies for *CYP2D6* alleles were similar to those described in other Mexican samples, we identified some differences (TABLE 2). In our study, the *CYP2D6* gene duplication frequency was 3.6% and was different to previous reports that estimated it in 12.76% in Mexican Mestizos [20], and 1% [22] and 0.8% in Mexican Americans [21]. A similar behavior was observed in the frequency of *CYP2D6*10*, that we estimated in 2.6%, and another report found it in 12.45% [20]. Our results suggest that these differences are not related to population structure, because we observed that the

Table 4. Frequency of *CYP2D6*82* allele in Mestizo and Amerindian Mexican populations.

Population (n)	Frequency (%)
Mestizo subpopulations	
Guerrero (50)	4.0
Yucatan (29)	3.4
Zacatecas (30)	1.7
Sonora (46)	0.0
Veracruz (29)	0.0
Guanajuato (26)	0.0
Amerindian subpopulations	
Mixtecos (30)	5.0
Tepehuanos (30)	3.3
Mayas (30)	1.7
Zapotecos (30)	0.0

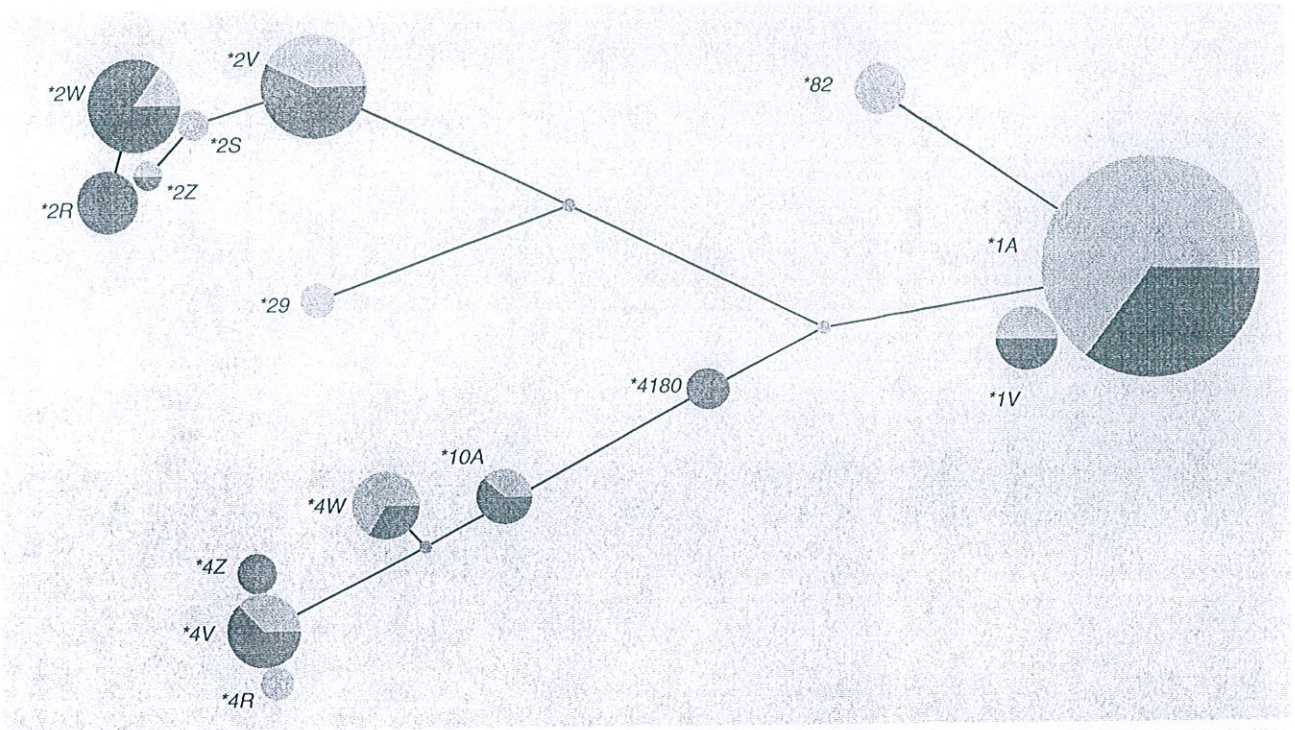

Figure 2. Network analysis of *CYP2D6* haplotypes. Circles represent haplotypes and its size is proportional to their frequency, light areas correspond to Guerrero and dark areas to Sonora. The line length between two haplotypes is proportional to mutational steps between them.

gene duplication and *CYP2D6*10* have similar frequencies to Mestizo subpopulations with ancestral contribution differences (SON and GUE), and they were very close to those of the Spanish population (Table 5) [54]. Thus, it is possible that these differences are related to issues regarding methodological approaches used in the allele reconstruction and/or determination. Although *CYP2D6*3* and *CYP2D6*17* have been observed in other Mexican populations with frequencies of approximately 1% or less, these alleles were not detected in our study probably owing to their low frequency. A similar situation may explain why we did not observe *CYP2D6*31*, a recently discovered low frequency (0.3%) allele in samples from self-defined Hispanics patients recruited in Kansas City, MO, USA [56].

It is known that *CYP2D6* allele frequencies vary between populations [42,45,46,54], and when we compared our results with those from other continental regions, we found clear differences between groups (Table 5). In Mexican Mestizos *CYP2D6*4* frequency is higher and significantly different to East Asians, such as Han Chinese [42] and Japanese [19,57]. An opposite trend is observed for *CYP2D6*10*, which has a low frequency in Mexicans and showed significant differences with Han Chinese and Japanese in which this is the main allele [19]. In our comparison, *CYP2D6*29* was only significantly different between Mexicans and African–Americans [46], and *CYP2D6*5* demonstrated significant differences between Mexicans and Han Chinese, African–Americans and Japanese. Interestingly, we observed similar frequencies of *CYP2D6*5* in Mexicans and populations of European descent, which suggests that this allele had similar frequencies in the two main ancestral populations of Mestizos (Amerindian and European). While few subjects with gene duplications are seen in Northern Europe, in the Mediterranean region approximately 10% of the population carry *CYP2D6* gene duplications [58]. Also, other studies have reported frequencies of 7.4% for Greek population [59] and 6.4% for Sephardic Jews [60]. The presence of *CYP2D6* duplications in Mexicans may be related to their European ancestral contribution, mainly Spanish and thus Mediterranean. The phylogenetic relationships among *CYP2D6* alleles that resulted from the network analysis (Figure 2), showed that the *CYP2D6*1A* is the most frequent allele in Mexican Mestizos and that some suballeles (**2S*, **4R* and **4Z*) are only observed in one particular geographic region correlating to either European or Amerindian

enriched subpopulations. These results show interesting coincidences with previous reports [19,42]; for example, haplotype **4180* derived from **1A* is only present in SON, and the SNP 4180G>C included in a haplotype previously reported [19] that has only been observed in Europeans and sub-Saharan Africans, this suggests that haplotype **4180* has a European ancestral origin in Mexican Mestizos. Also, the network analysis supports the hypothesis that two sets of new variants observed either in SON or GUE, may have arisen in a European (**2R* and **4Z*) or Amerindian (**2S* and **4R*) haplotypic backgrounds, respectively. All other variants have a similar distribution in both Mexican geographic regions and are similarly widely distributed in the world [19]; for them the potential ancestral origin cannot be defined.

Nucleotide sequences of *CYP2D6* and *CYP2D7* genes are 97% similar [6]. In general an efficient gene conversion requires homology between interacting sequences (usually >95%); and often results from genetic information transfer from nonfunctional pseudogenes to their closely related functional counterparts [61]. To date, several *CYP2D7* conversions have been reported, such as the ones involving intron 1, intron 4 or exon 9 [51,52,62]. We identified a novel *CYP2D7* exon 2 conversion, namely *CYP2D6*82* in GUE and we hypothesize an Amerindian origin for this variant. This is supported by the observation that the frequency of this allele in GUE (4.0%) was similar to those of two Amerindian groups included in the analysis, Mixtecos and Tepehuanos (5.0% and 3.3%, respectively), an also by the network analysis (FIGURE 2). CYP2D6 has a well-defined active site cavity bordered by the heme group and residues from the B′ helix, the B′-C loop and the F helix among others [63]. We hypothesize that three novel variants identified in *CYP2D6*82* could result in a protein functionally different to *CYP2D6*1* because the Val140Ala variant is flanking the B′ helix, and both Thr107Tyr and Ile109Val are inside of it.

A common assumption is that an allele would confer comparable activity in a given population. However, this assumption is not necessarily valid because unidentified sequence variations within *CYP2D6*, variations in other genes or gene-regulating regions, and/or nongenetic factors may contribute to modify CYP2D6 activity in different populations [46]. For example, we found

TABLE 3 Comparison of *CYP2D6* allele frequencies in Mexicans and other populations.

CYP2D6 alleles	MEX (n = 96)	Caucasians (n = 347) [45]		Han Chinese (n = 100) [42]		African–Americans (n = 272) [45]		Spanish (n = 105) [54]		Japanese (n = 162) [57]	
	Freq. (%)	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]	*Freq. (%)*	*p-value*[†]
**1*	50.0	36.46	<0.0001	18.5	<0.0001	32.90	<0.0001	31.0	<0.0001	39.8	0.015
**2*	25.5	21.90	0.168	14.0	0.003	13.98	<0.0001	40.47	0.001	12.3	<0.0001
**4*	14.0	19.74	0.043	1.0	<0.0001	3.86	<0.0001	13.8	0.528		<0.0001
**5*	2.0	3.31	0.270	7.0	0.017	6.43	0.012	3.33	0.325	6.2	0.023
**10*	2.6	2.16	0.444	49.0	<0.0001	2.94	0.510	1.9	0.444	37.0	<0.0001
**29*	1.0	0.14	0.120	0.0	0.239	7.54	<0.0001		0.227		0.138
**53*	0.5		0.217		0.490		0.261		0.478		0.372
Duplication	3.6	1.44	0.054	0.5	0.030	5.33	0.235	4.27	0.473	1.2	0.067

[†]*p-value for the comparison of allele frequencies between Mexicans and other populations.*
Freq.: Frequency.

novel variants of *CYP2D6*1*, *CYP2D6*2* and *CYP2D6*4* alleles with frequencies ≥3% (Supplementary Table 2), a novel *CYP2D7* exon 2 conversion, and a novel SNP in the 5′-flanking region of *CYP2D6* at position -801 in RefSeq (NG_008376.1) or 729 in M33388. A search for transcription factor-binding domains revealed that this novel SNP is within a putative site recognized by hepatic nuclear factor 4 (atagccc*gcCAGAgcccaggaatg) [64,65]. The presence of this variant in *CYP2D6* alleles may lead to the loss of putative sites recognized by HNF4 or affect the binding of regulatory proteins that could result in changes in the expression of this gene. The functional analysis of these alleles could help clarify specific relationships between the genotype and phenotype. Considering individuals (n = 5) with gene duplication of functional alleles (three active alleles), and those individuals (n = 3) with no active alleles in Mexican Mestizos, the frequencies of UM and PM phenotypes are expected to be 5.2 and 3.1%, respectively.

Conclusion

Our results demonstrate the utility to assay genetic variation in admixed populations to find novel variants in genes with known ethnical differences, such as *CYP2D6*, and gives information relevant for a comprehensive description of the genetic variability of this gene in Mexicans. This knowledge could be of critical relevance for the implementation of pharmacogenomics in Mexican Mestizos and related populations. To our knowledge, this study is the first comprehensive resequencing analysis of *CYP2D6* in Mexicans or any other Latin American population. An adequate assessment of CYP2D6 metabolic status considering intrinsic population characteristics before initiation of therapy, may improve patient outcome through the reduction of adverse drug reactions and by increasing drug efficacy.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- CYP2D6, a drug-metabolizing enzyme, shows a very high degree of interindividual variability that influences drug response and adverse reactions; its genetic variants play a critical role in interindividual differences affecting its expression and function.
- Individuals from two regions with different history of admixture within Mexico were resequenced to comprehensively characterize the *CYP2D6* gene in Mexican Mestizos, a recently admixed population resulting of the combination of Amerindian, European, and to a lesser extent, African populations.
- A total of 64 polymorphisms were found, including 14 novel variants: 13 SNPs and a *CYP2D7* exon 2 conversion, (*CYP2D6*82*); of these, three novel SNPs were predicted to have functional effects.
- Allele frequencies of *CYP2D6*1*, **2*, **4*, **5*, **10*, **29*, **53*, **82* and its duplication were, 50.0, 25.5, 14.1, 2.0, 2.6, 1.0, 0.5, 2.1 and 3.6%, respectively, and significant frequency differences between Mexicans or other population groups were found.
- For the novel *CYP2D6*82* an Amerindian origin was hypothesized and supported by its identification in three Mexican Amerindian groups (Mayas, Tepehuanos and Mixtecos).
- The functional analysis of novel potentially functional alleles could help clarify specific relationships between genotype and phenotype.
- Considering the identified number of individuals with gene duplications of functional alleles and with deletions, the frequencies of ultrarapid and poor metabolizer phenotypes in Mexican Mestizos are expected to be 5.2 and 3.1%, respectively.
- Strong linkage disequilibrium and phylogenetic relationships between haplotypes were observed in genotypic data analyzed.
- An adequate assessment of CYP2D6 metabolic status considering intrinsic population characteristics may improve patient outcome through the reduction of adverse drug reactions and increasing drug efficacy.

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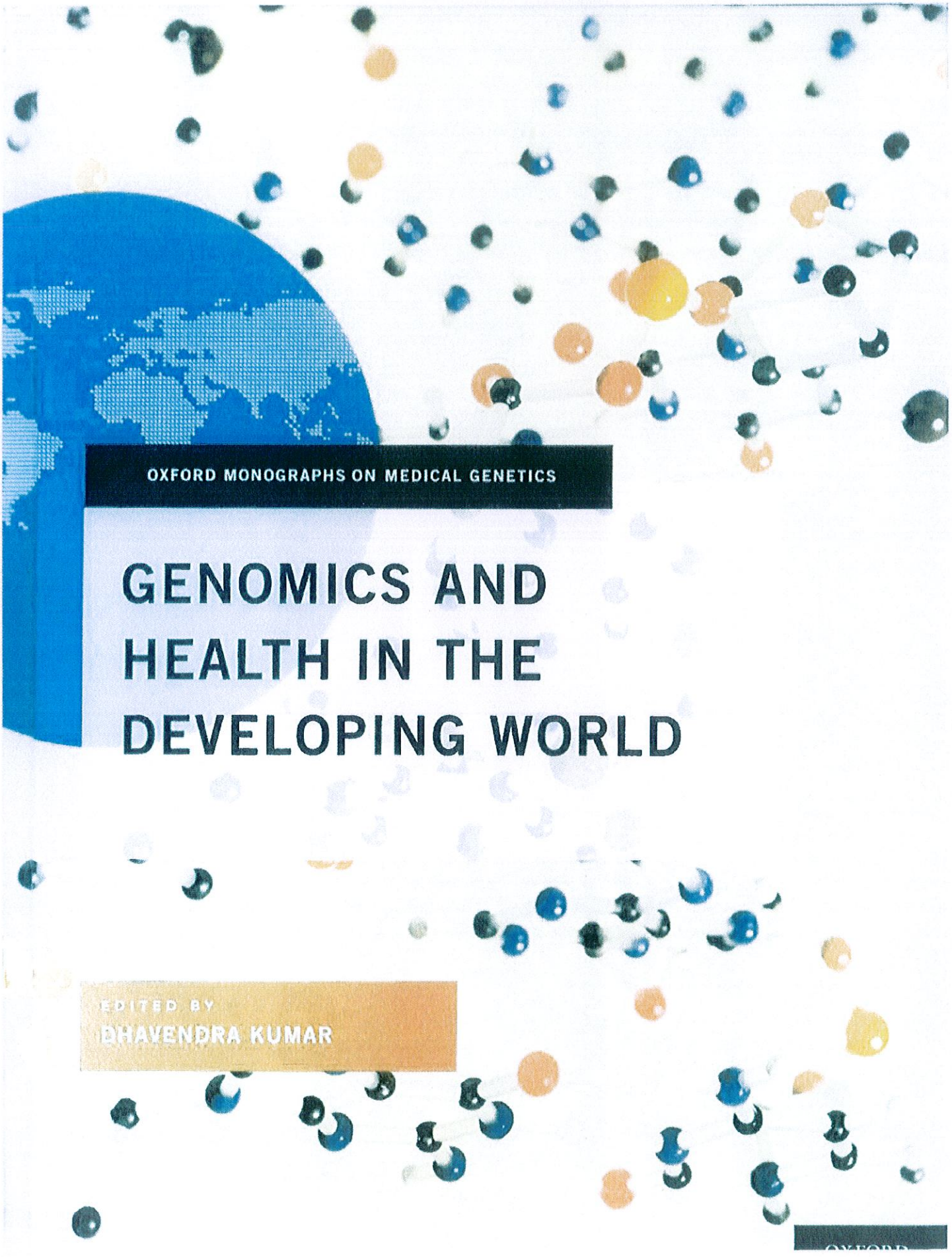

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GENOMICS AND HEALTH IN THE DEVELOPING WORLD

EDITED BY
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DESIGN AND IMPLEMENTATION OF A PLATFORM FOR GENOMIC MEDICINE IN MEXICO

Gerardo Jiménez-Sánchez, Julio Frenk, and Guillermo Soberón

INTRODUCTION

The spectacular progress in biochemistry, molecular biology, genetic engineering, and biotechnology that occurred during the second half of the twentieth century paved the way for the sequencing and mapping of the human genome. Genomes contain all of the information needed to determine the functions of an organism and maintain homeostasis, a state of equilibrium within an organism's internal environment. The healthy state depends on the correct functioning of the genome in interaction with the environment. Because of these homeostatic systems, organisms can dynamically adapt to changes in the environment. When this equilibrium is disrupted, disease occurs.

The Human Genome Project (HGP) represented a milestone in human history, not only because of its scientific and technological scope but also because of the great impact that this information will have on people's daily lives. Knowing how variations in the genome confer human individuality will be of great use in medicine, giving physicians the ability to prevent, diagnose, and treat common diseases (Childs et al., 2001). Moreover, the rise of genomic sciences has propelled the development of new technologies to sequence and study the human genome and that of many other species. These technologies have led to novel and more sophisticated opportunities for economic growth through the development of new products, the creation of new intellectual property, commercialization, and identification of new markets. However, these discoveries also pose important ethical, legal, social, political, and even religious challenges that demand adaptive changes in contemporary societies. Thus, it is of great importance that all members of society understand the opportunities and risks that this information generates, so that necessary actions can be implemented to allow equal access to medical applications while preventing inappropriate use of information, thus complying with universal principles related to human rights and respect for societal values (Jiménez-Sánchez, 1999).

The analysis of the results from the HGP has increasingly made evident important differences between traditional genetics, which studies individual genes or relatively small groups of genes, and genomics, which is dedicated to the comprehensive study of genomes with a global focus that covers all the genes in a species. Research in this field has become increasingly focused on understanding the function and regulation of each gene and its interactions with other genes and with the environment.

In February 2001, the first "draft" of the human genome was published. The results of the HGP, supported by the United States government, were published in *Nature* (Lander et al., 2001). Simultaneously, the sequence resulting from the private project by Celera Genomics was published in *Science* (Venter et al., 2001). In addition to mapping and sequencing of the human genome, these projects have produced the first analysis of the genome's contents. Thus, to systematically identify genetic variations in the human genome, a new project was developed: the "HapMap." Its main goal was to describe the most frequent genetic variations in the genome of three ancestral populations: European, Asian, and African. This project successfully concluded in 2006, providing a series of fundamental tools to contribute to the identification of the genetic bases of common diseases (HapMap, 2005). This and other subsequent projects, including ENCODE and "The 1000 Genomes" (Kaiser, 2008), have significantly improved our knowledge of genetic variation and have contributed to the identification of disease-associated genes.

The human genome contains variations in its sequence, the combinations of which confer individuality to each member of the human species. All the members of our species share around 99.8% of our genome. However, there are different kinds of genetic variation in the human genome. Among these, there are between 3 to 10 million single nucleotide polymorphisms. If we calculate the number of possible combinations of these, and other variations also available in the human genome, it will become

clear why each individual has his or her own genetic combination, accounting for about 0.1% of the total sequence. The ability to uniquely identify each individual will have applications in different areas. In fact, in some countries, DNA banks are being developed to identify individuals based on currently known polymorphic variants.

In addition to the complete sequence of the human genome, a map that contains around 23,000 genes and a catalog of more than 1200 genes associated with diseases (Jiménez-Sánchez et al., 2001a) and genetic variations were published. These variations include single nucleotide polymorphisms (SNPs), copy-number variations (CNVs), duplications, and deletions (Iafrate et al., 2004; Wakeley et al., 2001).

IMPLICATIONS FOR HEALTHCARE

Genomic medicine, defined as the analysis of genetic variation to identify risks for common diseases, will lead to a more individualized, more predictive, and more preventive medical practice based on the genetic protection or risk conferred by each individual's genome. Thus, to develop specific applications to diagnosis and treatment of human disease, it is necessary to identify genetic variants associated with common disease risks (Jiménez-Sánchez et al., 2001a).

Our ability to analyze the whole human genome and associate genetic variations to common traits makes an important distinction relative to traditional human genetics approaches, although both disciplines can complement each other to achieve their own goals. Applications derived from the human genome will offer new forms of prevention, including the ability to develop presymptomatic identification of individuals at risk. This may lead to new strategies to influence individuals' lifestyles as part of their primary healthcare.

Overall, genomic medicine has the potential to prevent or delay the presence of common diseases in humans. As a result, it is not too bold to anticipate that, within a few years, clinical medicine will be increasingly immersed in the interpretation of genomic findings and in the design of programs to optimize healthcare of individuals based on those findings.

One of the benefits derived from genomics and proteomics is the ability to design new pharmaceutical drugs based on the identification of their molecular targets and their regulatory mechanisms. Furthermore, it will make it possible to identify those individuals likely to have unusual responses to certain drugs, leading to a safer use of them (Jiménez-Sánchez et al., 2002c). Thus, pharmacogenomics studies the response of individuals to pharmaceutical agents based on the characteristics of their genome (Evans & Relling, 1999; Emilien et al., 2000; Sadée, 1989). Its development potentially will lead to the design of products directed to population groups that share specific DNA sequences associated with better medical effects and less toxicity.

ETHICAL, LEGAL, AND SOCIAL IMPLICATIONS

The use of genomic information may have implications of great individual and social relevance. There are important ethical, legal, and social issues related to the use of genomic information, which contemporary societies need to address in a timely way (Jiménez-Sánchez & Lara Alvarez, 2007). Some of the most relevant challenges include prevention of stigmatization and discrimination of individuals based on their genomic makeup. This includes fair use of genetic information by insurance companies, employers, the legal system, schools, and other institutions. It is therefore important to develop regulatory frameworks that regulate access to this information, among other issues.

Thus, it is essential that the study of the human genome and the development of medical applications be undertaken under ethical principles that will allow societies to benefit from these new opportunities, as well as to avoid the risks of misuse, ensuring respect for the rights and dignity of individuals.

UNESCO produced a "Universal declaration on the human genome and human rights" during the 29th meeting of its General Assembly in November 1997. This declaration, unanimously approved by 186 United Nations member countries, indicates that "no research or research applications concerning the human genome...should prevail over respect for the human rights, fundamental freedoms and human dignity of individuals or, where applicable, of groups of people."

Research on this topic has received worldwide attention. In Mexico, investigators from the Center for Interdisciplinary Studies on Health and Law (http://www.juridicas.unam.mx/invest/areas/neisd/) at the Research Institute of the National Autonomous University of Mexico (UNAM) have worked on different aspects of the legal and social implications of genomic research and have made important contributions to this field (Muñoz de Alba, 2002).

In addition, Mexico has a National Commission of Bioethics that analyzes such challenges in the context of universal principles and national legal frameworks. This commission acquired the status of a decentralized agency of the Mexican Federal Government in September 2005. Recently, it has been provided with growing resources to undertake an ambitious program, which includes studying the ethical, legal, and social aspects of genomic medicine.

HUMAN GENETICS IN MEXICO

Mexico has a solid tradition of biomedical and health sciences, upon which it is possible to construct the basis for

genomic medicine. The disciplines of human genetics, biochemistry, molecular biology, microbiology, immunology, biotechnology, neuroscience, pediatrics, internal medicine, and geriatrics are all mature, and they constitute indispensable elements to the development of genomic medicine research and its applications to public health.

The study of human genetics in Mexico is a relatively recent development; it was only at the end of the 1940s that the first publications of Dr. Mario Salazar Mallén described the distribution of blood types among the Mexican population. In the 1960s, the first genetic research groups were established in hospitals both in Mexico City and Guadalajara. Soon after, the Mexican Association of Human Genetics emerged, along with human genetics as a specialty of medicine at the National Medical Center of the Mexican Institute of Social Security (IMSS) and the Mexican Board of Human Genetics.

Since its foundation in Mexico, human genetics has achieved significant progress in different areas, such as clinical practice, cytogenetics, population genetics, biochemical genetics, neonatal screening and, more recently, molecular genetics. The most relevant contributions of Mexican geneticists are discussed in three publications compiled by Kofman-Alfaro et al. (1991), Salamanca and Armendares (1995), and Lisker and Carnevale (1995). The groups that were progressively established during the last decades have made contributions to scientific research, healthcare, and higher education.

Human genetics in Mexico is a highly decentralized discipline resulting from the establishment of groups of geneticists across the country. Most of these scientists have made important contributions for which they have received international recognition. In the last years, not only have clinical geneticists been trained, but also doctoral programs have produced graduates in the field.

GENOMIC SCIENCES IN MEXICO

During the last decade, the Mexican government has strengthened its commitment to improving competitiveness and innovation through science and technology. Although investment in this area has been consistently limited, from 1995 to 2005 the percentage of the gross domestic product (GDP) devoted to science and technology increased from 0.35% to 0.43%. The number of students registered in doctoral programs in science and technology increased from 488 in 1994 to 2009 in 2005. From 2001 to 2005, the number of researchers in the National System of Investigators increased by 62% (Triunfol, 2007). In addition, in 2002, the Chamber of Deputies of the Mexican Congress approved a new Law of Science and Technology, through which new funding sources, known as the Sectorial Funds for Science and Technology, were established. These funds are composed of equal contributions from the different secretaries (departments), states, districts, and the National Council of Science and Technology (CONACYT).

Mexico currently has more than 12,100 researchers registered in the National System of Investigators. Among them, 24.5% are established at UNAM, and the rest are at public state universities throughout the country. In medical research, the National Institutes of Health (NIH) have about 500 researchers (4.1%), the Mexican Institute of Social Security has around 294 (2.4%), and other areas in the health sector have 87 researchers (0.7%). The fields of biology, chemistry, medicine, and health sciences contribute with 26.7% of the total, reflecting a sustained increase during the past ten years (CONACYT, www.siicyt.gob.mx). Overall, the number of investigators continues to increase, as well as the number of areas in which they work.

In the past 15 years, Mexican investigators have made initial contributions to genomic sciences. These include participation in the genome project for *Escherichia coli* (Blattner et al., 1997) and the first large-scale sequencing project in Mexico, the sequencing of the genome of *Rhizobioum etli*, conducted by the Center of Genomic Sciences (CCG; www.ccg.unam.mx), formerly known as the Center for Research in Nitrogen Fixation (CIFN; Gonzalez et al., 2006), at UNAM. These and other projects have led to a series of genomic initiatives related to different organisms, including the parasite *Taenia solium*, whose genome is also currently being sequenced at UNAM (Aguilar-Diaz et al., 2006). Other projects include the sequencing of plant species, which is mainly being undertaken at the Center of Research and Advanced Studies (CINVESTAV) of the National Polytechnic Institute (www.cinvestav.mx). Recently, the National Laboratory of Genomics for Biodiversity (LANGEBIO) was created with the goal of carrying out important genomics projects in plants, such as the sequencing and functional analysis of the genome of maize. Additional infrastructure for genomic research is located in the states of Guanajuato, Nuevo León, Jalisco, Tamaulipas, and Yucatán, among others (Jiménez-Sánchez et al., 2008).

In 2004, an undergraduate program in genomic sciences (LCG) began at UNAM (www.lcg.unam.mx) at the CCG and the Biotechnology Institute in Cuernavaca, Morelos. The curriculum includes intensive education in mathematics, statistics, computational sciences, and biology (Palacios & Collado-Vides, 2007). In recent years, two new scientific societies have been founded: the Mexican Society of Genomic Sciences (http://smcg.ccg.unam.mx/) and the Mexican Society of Genomic Medicine (www.somegen.org.mx), with the latter headed by Gerardo Jiménez-Sánchez as its founding president. These societies gather together the majority of scientists who work in the field of genomics in Mexico.

FROM HUMAN GENETICS TO GENOMIC MEDICINE

Mexico has a long tradition in the biomedical sciences and health. Currently, there are more than 250 professionals registered with the Mexican Association of Human Genetics, and more than 175 certified geneticists registered by the Mexican Board of Human Genetics. A variety of institutions together offer more than 50 formal, independent graduate programs in human genetics and molecular biology. As a result, independent research groups have been established in areas that include clinical genetics, cytogenetics, genetic epidemiology, population genetics, inborn errors of metabolism, neonatal screening, genetic toxicology, and molecular genetics (Salamanca & Armendares, 1995).

Historically, the majority of important contributions in genetic research by Mexican-Americans have occurred outside of Mexico, and some contributions have resulted from the collaboration with Mexican researchers. In Mexico, the majority of studies related to the genetic basis of complex diseases have primarily analyzed known variants in candidate genes. Initially, such studies included the association of the HLA-B27 allele with ankylosing spondylitis (Fraga et al., 1979) and the association of other HLA alleles with rheumatoid arthritis. Since then, researchers have described associations between HLA alleles and various complex diseases common among Mexicans. These diseases include type 2 diabetes mellitus and some of its complications (Perez-Luque et al., 2003), generalized lupus erythematosus (GLE; Bekker-Mendez et al., 1998), scleroderma (Vargas-Alarcon et al., 1995), and type 1 diabetes mellitus (Gorodezky et al., 1995).

Other complex diseases have also been studied using candidate gene approaches. Some examples include the analysis of SNPs in the genes encoding insulin, and others associated with different abnormalities in serum lipid levels (Sanchez-Corona et al., 2004), such as hepatic nuclear factor 4-alpha (Weissglas-Volkov et al., 2006), tumor necrosis factor-alpha (Parra-Rojas et al., 2006) and *ABCA1* (Villarreal-Molina et al., 2007). In addition, there have been associations described between GLE and polymorphisms in *PTPN22* (Baca et al., 2006) and *PCDC1* (Velazquez-Cruz et al., 2007) in children, as well as between GLE and polymorphisms in *PCDC1* in adult patients (Prokunina et al., 2002).

The 677C>T polymorphism in the methylenetetrahydrofolate reductase gene (*MTHFR*) is associated with diseases related to hyperhomocysteinemia and folate deficiency. It has been found that, among Mexicans, the 677C>T allele is weakly associated ($p = 0.05–0.01$) with hyperhomocysteinemia (Torres-Sanchez et al., 2006), anencephaly (Munoz et al., 2007) and gastric cancer (Lacasana-Navarro et al., 2006).

The analysis of genetic variations related to pharmacological responses has mainly focused on the genes that encode for metabolizing enzymes, such as *CYP2D6* (Contreras et al., 2011; Hidalgo-Miranga & Jiménez-Sánchez, 2009). In the case of this gene, the allelic frequencies show significant differences among the different populations of Mexico. For example, *CYP2D6*10* has a frequency of 12.45% among individuals in Mexico City (Lopez et al., 2005) and a frequency of only 2.3% among individuals in the north-central state of Durango (Sosa-Macias et al., 2006).

Clearly, Mexican geneticists have achieved important milestones; however, to identify novel genes associated to common diseases in Mexicans, it is important to implement modern research strategies such as genome-wide association studies (GWAS), dense genotyping, and resequencing approaches, among others, to achieve an emergence of research in genomic medicine.

Genomic analysis of cancer in Mexico is currently undergoing a transition from the classic genetic approaches to whole-genome analysis, including whole expression and copy-number variations analyses (Hidalgo et al., 2005; Valladares et al., 2006; Vazquez-Ortiz et al., 2007). This is transforming the area of molecular oncology in Mexico, particularly in cervicouterine and breast cancers.

To further develop human genetics and genomic medicine in Mexico, new experimental designs are required, including those considering the genetic structure of the Mexican population, supported by the collection of large numbers of disease-specific samples. In addition, training a higher number of health professionals in genomics and public awareness programs are needed so that Mexico takes full advantage of genomic medicine.

DEVELOPMENT OF GENOMIC MEDICINE IN MEXICO

Genomic medicine will allow for more individualized, thus effective, interventions. These will need to be incorporated into health policies to turn medical practice into a more individualized, predictive, and preventive discipline. Thus, scientific research must be a priority to identify disease-associated genetic variants in the Mexican population. Currently, an important number of genetic variants have been identified for various common diseases, including diabetes mellitus (Wellcome Trust, 2007; McCarthy, 2010), cardiac diseases (Wellcome Trust, 2007), Crohn's disease (Mathew, 2008), and various types of cancer (Easton et al., 2007; Zanke et al., 2007). This information, and its corroboration in different populations, has the potential to strengthen screening programs, diagnostic and prognostic strategies, and the identification of new therapeutic targets and novel molecules of pharmacological use. Such strategies would not only decrease chronic complications and their associated costs, but can also

improve medical care and the patient's quality of life, and lead to a more rational use of health resources and better-supported decisions in public health (Jiménez-Sánchez et al., 2001b).

Many believe that the main challenges that Mexico faces can be divided into two categories: on one hand, overcoming painful lags in development that work against the dignity of its people, and on the other, the capacity to develop strategies to join emerging opportunities at the frontier of science and technology for the benefit of its people.

The challenges that a country must face in relation to its economic and social development are many and varied. There are pressing problems that will be overcome in the midterm and long term future. However, we have learned that challenges should not be dealt with in chronological order. Thus, it is not valid to suggest that Mexico should not embrace the emerging frontiers of science and technology to improve healthcare and wellbeing until previous, pressing challenges are solved. In fact, overcoming current hurdles and embarking upon promising new paths should be done simultaneously. As such, it is universally recognized that economic and social development must be pursued simultaneously.

In the case of healthcare, it is a false dilemma to put problems of underdevelopment such as malnutrition, infections, and pathologies related to reproduction, against the important opportunities that emerge in biotechnology, information technologies, and telecommunications—which can translate into greater possibilities for the diagnosis, treatment, control, and prevention of common diseases. This means that the Mexican health system should continue to improve its programs by incorporating new cost-effective interventions, increasing coverage for services that are already nearly universal, strengthening primary healthcare programs, promoting community medicine, and developing a health culture based in education. Furthermore, the National Health System should have the means to identify, evaluate and, when necessary, implement the rapidly emerging technological innovations that can strengthen healthcare in a variety of ways.

These thoughts were included in the 2002 declaration at the World Health Organization stating that Mexico cannot afford to be indifferent and passive about genomic medicine. The potential benefits that genomic medicine offers are significant, and it is absolutely essential that these opportunities be considered as part of the national health programs (Jiménez-Sánchez, 2003).

At that time, it was clear in Mexico that genomic medicine will translate into a new paradigm for healthcare, with a number of opportunities that would represent important benefits for the Mexican population. In addition, the solid infrastructure in genetics and clinical medicine, along with the enthusiasm to develop genomic sciences in Mexico, represented significant assets that could serve as the basis to develop genomic medicine. Moreover, it was recognized that the development of genomic medicine would require the analysis of genetic variations in the Mexican population, as well as a robust human and technological infrastructure to support/facilitate research and implementation programs. Thus, genomic medicine clearly was not an "out of the box one-fits-all" solution.

The available evidence indicated the important benefits that genomic medicine could represent for Mexico, as well as the risks if such development was delayed. These included lack of access to novel knowledge and applications to public health, and the risk of increasing Mexico's dependence on developed economies in sensitive areas such as health. This led to the decision of incorporating genomic medicine as a part of Mexico's National Health Plan. As a result, it stimulated the development of cutting-edge infrastructure to implement world-class genomic medicine, oriented to the development of early interventions for prevention, diagnosis, and treatment of common diseases such as hypertension, type II diabetes mellitus, asthma, acute myocardial infarction, and a number infectious diseases that are becoming increasingly relevant in Mexico as part of the epidemiological transition in progress (Jiménez-Sánchez, 2003). The sustained development of this strategy would lead to improvement of healthcare in Mexico, and makes it reasonable to predict an important economic impact related to the reduction of health-related costs, mainly those of chronic treatment and loss of productivity from individuals with common diseases (Jiménez-Sánchez et al., 2002a).

A major component of this strategy was oriented toward educational programs aimed at professionals and the general public. The development of a national platform in genomic medicine will lead to the creation of infrastructure to develop training programs at different levels. In addition, such a strategy would lead to new opportunities to increase participation in a knowledge-based economy, including the stimulation of the genomic industry as a means to contribute to the economic development of Mexico (WHO, 2002). Although the costs of this initial infrastructure seemed high, they were relatively moderate compared with the financial costs that result from the burden of disease or the dependence on other countries with expertise in such a significant strategic area.

Mexico has extraordinary opportunities to identify genetic variants associated with medical traits, due to the availability of isolated populations, which are highly inbred and genetically homogenous. We suggested that international collaborations should be stimulated; however, abusive relationships must be avoided in which samples from Mexicans are taken to more industrialized countries, impeding access to research and results. This has occurred in the past in the field of archaeology and in the study of Mexican fauna and flora. For these reasons, it is strategically important that Mexico takes advantage

of the new knowledge and technologies generated by the HGP. To do this, it will be necessary to train a critical mass of researchers, professionals, and technicians who have the capacity to assimilate, develop, and apply genomics to medicine (Jiménez-Sánchez et al., 2008).

ANCESTRY OF THE MEXICAN POPULATION

Mexico is the fourteenth largest country in the world, with a total area of 1,972,550 km^2 and close to 112 million inhabitants (INEGI, 2005). Its varied topography results in a variety of climate conditions, from arid deserts in the north to rainy tropical climates in the south and along the southeast coast. In addition, geographically distant regions have different demographics. These differences arise from both the country's distinct ancestral components and from the demographic conditions that characterize each region (Gerhard, 1986). During the pre-Hispanic era, the majority of the population was concentrated in the center and south of Mexico. The ethnic groups that inhabited the north of Mexico did not have linguistic, religious, or political unity. It was not until two centuries after the Spanish conquest that the northern regions drew the attention of the Spaniards, mainly because of the silver deposits discovered in those regions (Gerhard, 1986). After the notable reduction of the Amerindian population as a consequence of epidemics between 1545 and 1548, African slaves were brought into Mexico. These slaves mixed both with indigenous inhabitants and with Mestizos, and many were transferred to other regions to work in mineral mines. The Yucatan Peninsula, located in the southeast region of Mexico, was populated by different Amerindian groups who were decimated by diseases, which reduced the original population by at least one-half (Gerhard, 1991). This unique history translates into a population that is derived from more than 60 groups of local Amerindians, Europeans, and, to a lesser degree, Africans. A survey of the language and geographic location of Amerindian groups in Mexico is currently being conducted. These groups have mixed throughout the past 500 years, leading to the Mestizo population that currently represents 80% of Mexicans (Gonzalez Burchard et al., 2005). Because of this unique demographic history, it is important to characterize the genetic composition of the Mexican population as an initial step toward the successful development of genomic medicine in Mexico.

STRATEGY TO ESTABLISH A NATIONAL INSTITUTE OF GENOMIC MEDICINE

As is the case for the majority of developing countries, Mexico faces demographic and epidemiological transitions that have important implications for the standards of disease, disability, and mortality. On one hand, it faces the unresolved problems of infections, malnutrition, and reproductive health, and on the other, the emerging challenges of chronic and degenerative diseases of the industrialized world. In Mexico, the adult population has a high prevalence of diabetes mellitus (7.0%), hypertension (30.8%), and obesity (29.4%; Olaiz, 2006). The two main causes of mortality are cardiovascular diseases (22.9%) and diabetes mellitus (15.3%; INEGI, 2005). Preventing these diseases is a key strategy to reducing their significant economic and health burden.

In 2000, an initial working group analyzed reasons why genomic medicine represented important opportunities for healthcare in Mexico. The group identified the following eight areas of opportunity: (1) contribution to a more individualized, predictive, and preventive medical practice; (2) strengthening of scientific research and technology in Mexico; (3) potential cost reduction of healthcare; (4) development of pharmacogenomics; (5) generation of novel products and services; (6) strengthening of the potential to participate in the knowledge economy; (7) timely development of an ethical and legal framework for genomic medicine in Mexico; and (8) the development of public education programs related to genomics and society.

The establishment of the National Institute of Genomic Medicine was preceded by multi-institutional efforts aimed at developing a national platform for genomic medicine in which this institution would have a central role. In 1999, the Mexican Health Foundation (FUNSALUD), then under the leadership of Dr. Guillermo Soberón, organized a working group composed of specialists from the NHIs and UNAM. The group analyzed the state of the art of genomic sciences at that time, as well as the possibilities to use such progress to improve healthcare for the Mexican population.

Simultaneously, the federal government of Mexico, then headed by President Ernesto Zedillo, began to generate the infrastructure that Mexico would need to coordinate public policies and actions regarding the human genome. In consequence, José Antonio González Fernández, Secretary of Health at that time, established the National Commission for the Human Genome on October 23, 2000. This organism was created by the action of an executive decision of the President of Mexico, which was co-signed by the secretaries of health and education. This Commission provided an ideal forum for discussion at the highest levels of decision making, and was instrumental in speeding the process of developing genomic medicine in Mexico.

In 2000, a strategic alliance was established to generate synergies for the project. This alliance was integrated by the Department of Health (SSA), UNAM, CONACYT, and FUNSALUD. Its goal was to evaluate the best way to create an institution that would coordinate the development of

a national platform of genomic medicine. On August 27, 2001, with the assistance of specialists from the McKinsey consulting firm, this group produced a feasibility study to launch the initial steps for a national platform of genomic medicine in Mexico. This study recommended the creation of a Consortium to promote and perform executive and detailed studies, in order to create and develop the first National Health Institute in Latin America dedicated to genomic medicine, while the Mexican Congress analyzed a bill to create the National Institute of Genomic Medicine (INMEGEN). As a result, the Consortium for the Institute of Genomic Medicine was established on November 22, 2001.

The Consortium was designed with a very specific goal, namely to promote and perform executive and detailed studies to establish and develop INMEGEN (Jiménez-Sánchez et al., 2002b). Its organic structure was headed by a governing board, integrated by Julio Frenk, Secretary of Health; Juan Ramon de la Fuente, Rector of UNAM; Jaime Parada Avila, Director of CONACYT; and Antonio Lopez de Silanes, President of the Board of Directors of FUNSALUD. From it, a board of directors was formed that included representatives from each of the institutions of the alliance: Misael Uribe (SSA), Alfonso Serrano Perez-Grovas (CONACYT), Juan Pedro Laclette (UNAM), and Guillermo Soberón (FUNSALUD), along with a fifth person, Dr. Jorge Rosenkranz, who was included because of his experience in the fields of research and the industrial sector. Guillermo Soberón was appointed coordinator of this board. Subsequently, they elected Gerardo Jiménez-Sánchez as director of the Consortium, and Cuauhtemoc Valdes from FUNSALUD was invited to take care of administrative coordination.

The Consortium existed from January 2002 until May 2005, when it had successfully achieved its goal. The director of the Consortium periodically presented the work programs and reports for the consideration of the board of directors. The board monitored and supervised the activities and financial status. Furthermore, the Board of Governors was kept informed on an ongoing basis, and periodic meetings were held to formally present the appropriate progress reports.

Given the novel nature of genomic medicine, the Consortium dedicated part of its efforts to broadly disseminate information on the nature and the scope of the project to the academic community and the general public. Throughout the project, more than 100 lectures were held. Many of them were offered at the most distinguished academic institutions, including El Colegio Nacional, the National Academy of Medicine, and the Industrial's Club, the latter aimed at the business community. These conferences were delivered both by domestic and foreign speakers such as Barton Childs, Francis Collins, Aravinda Chakravarti, David Vaile, Rick Ward, Julio Frenk, Juliana Gonzalez, Gerardo Jiménez-Sánchez, and Guillermo Soberon. In addition, the first National Congress of Genomic Medicine was celebrated in 2004 with participation of top international academic leaders in genomic medicine.

Antonio López de Silanes, a prominent businessman in Mexico and, at that time, president of the Board of Directors from FUNSALUD, organized a group of 37 FUNSALUD associates with experience in the pharmaceutical industry, health, and insurance services, along with other people interested in the topic. This group made it possible for FUNSALUD to contribute to the trust of the Consortium. Additionally, some companies (Laboratorios Silanes, GlaxoSmithKline, Merck Sharp & Dohme, and Novartis) provided support for specific projects and actions of the Consortium. One example of such support was the establishment of the Silanes-INMEGEN Award in genomic medicine in 2002, and the Silanes-FUNSALUD Chair of Genomic Medicine in 2004.

Since then, FUNSALUD has been disseminating information about genomic medicine on its own, as well as through joint events with the National Academy of Medicine and through written reports published in the *Gaceta Medica de Mexico*. An edition of *Cuadernos FUNSALUD* was dedicated to reviewing the achievements of genomic medicine and the opportunities for the pharmaceutical industry.

As has been mentioned, the Consortium was in charge of formulating the distinct projects and documents that served as the basis for the discussion of this initiative with the legislators at that time, mainly with those in the health and science and technology committees from each of the two chambers of the Mexican Congress, as well as with the Department of the Treasury to lay the foundations of the financial feasibility of the project.

The Consortium produced a number of national congresses, seminars, and courses. Among those, Gerardo Jiménez-Sánchez designed three courses that integrated genomic medicine and clinical practice; namely, "introduction to genomic medicine," "pediatric applications of genomic medicine" and "genomic applications in internal medicine," which were registered with the Faculty of Medicine at UNAM. In addition, an electronic portal was established to identify potentially interested candidates to pursue a career in the field. This portal produced extraordinary results: an enormous interest was seen among young doctors, biologists, chemists, engineers, and other professionals, many of them Mexicans receiving graduate training abroad. Several of them subsequently joined INMEGEN.

The research activities soon began, and it became necessary to construct an adequate space for the laboratory work and for highly specialized technological units. We established the initial headquarters of the Consortium on a 3000m² area at the Zafiro corporate tower in south Mexico City. In this space, we established

the first laboratories and the following three core centers: sequencing, microarrays, and high-performance computing.

The results obtained by the Consortium were published in two separate reports (Jiménez-Sánchez, 2002, 2004b), which were approved by the Board of Governors and the Board of Directors of the Consortium. They were made publicly available and widely distributed to the institutions and public in Mexico.

THE FOUNDATION AND INITIAL DEVELOPMENT OF INMEGEN

In Mexico, the law of the National Institutes of Health indicates that NIHs are to be created by the Mexican Congress. The work done by the Consortium laid the foundations for discussion in the Mexican Congress regarding the need for the National Institute of Genomic Medicine. Thus, the process that began at the end of 1999 concluded in April 2004, following a significant number of actions to promote such legislation. On July 19 of the same year, a decree was published creating the National Institute of Genomic Medicine, in a ceremony headed by then President of Mexico, Vicente Fox, and in which then Secretary of Health, Julio Frenk, and the president of the Health Commission of the Chamber of Deputies, Jose Angel Cordoba Villalobos, participated. It is worth mentioning that Dr. Cordoba Villalobos followed Julio Frenk as Secretary of Health on December 1, 2006.

During the administration of President Felipe Calderon, INMEGEN received a visit from the First Lady of Mexico, Ms. Margarita Zavala, who inaugurated the first three High-Technology Core Units on the third anniversary of the founding of the institution. Later, on May 11, 2009, during the presentation of the results from the Mexican Genomic Diversity Project, the President of Mexico expressed his support of INMEGEN and urged it to continue working toward full development of genomic medicine in Mexico.

According to the Law of the National Institutes of Health, INMEGEN is the National Reference Center for matters related to the human genome and its medical applications. It has the following main objectives: (1) to perform clinical, epidemiological, experimental, technological, and basic studies and research in its areas of specialty for the understanding, prevention, diagnosis, and treatment of diseases and the rehabilitation of the ill; (2) to promote public health measures; and (3) to perform activities inherent to the National Institutes of Health, except for those related to providing medical services. The latter is relevant because in Mexico, all NIHs except for two (Public Health and Genomic Medicine) are highly specialized hospitals in addition to research institutions, whereas INMEGEN was planned as an institution where clinical research would be carried out using the clinical infrastructure of the rest of the NIHs. This feature, called the *horizontal dimension*, was designed during the feasibility study to develop robust synergies without diluting INMEGEN's research budget. In addition, the Institute was tasked with promoting projects to develop specialized technology based on specific projects involving technological innovation.

The same law defines the administrative bodies of the Institute. Thus, the Governing Board was established for the period from 2004–2009, in accordance with Chapter III (dedicated to the governing bodies), Article 14 of the cited law. From the first to the fifth years, the Governing Board consisted of the Secretary of Health, who presided over it; the General Coordinator of the National Institutes of Health; a representative of the Department of the Treasury; the President of the Board of Trustees of the Institute; a representative of the educational sector; and four invited members designated by the Secretary of Health from other institutions who were recognized for their moral quality, merits, prestige, and experience in their areas of expertise. These last members are invited for four years, with the possibility of being reappointed for one additional term; however, none of them were reappointed by the following federal administration. In addition, the Governing Board had a secretary and a deputy secretary.

During 2004, this Governing Board was presided over by the Secretary of Health, Dr. Julio Frenk Mora, and was composed of the following members: Dr. Jaime Sepulveda Amor, Deputy Chairman and General Coordinator the National Institutes of Health; Dr. Guillermo Soberon, President of the National Commission of Bioethics; Dr. Manuel H. Ruiz de Chavez, Executive President of the Mexican Health Foundation; Dr. Juliana Gonzalez Valenzuela, Emeritus Professor of the Department of Philosophy and Arts of UNAM; and Dr. Jorge Rosenkranz Weiner, distinguished Mexican scientist with special expertise in intellectual property in biotechnology; Mr. Sergio Montaño Fernandez, representative of the Department of Treasury, and Dr. Rene Santoveña Arredondo, representative of the educational sector and Rector of the Autonomous University of the State of Morelos; Mr. Carlos Eduardo Represas de Almeida, President of the Board of Trustees and Chair of the Governing Board of Nestlé Mexico, S.A. de C.V.; and Sergio Vazquez Cordova, as Commissary.

The members mentioned above formed the Governing Board until 2006. With the change of federal administration, beginning in 2007, Dr. Jose Angel Cordova Villalobos, Secretary of Health, presided over the organization, and Dr. Julio Sotelo Morales joined as Alternate Chairman and Trustee of the Coordinating Commission of the National Institutes of Health and High-Specialization Hospitals.

Several other members of the board changed, including the four invited members named.

In September 2008, given that no members were reappointed upon concluding their terms, the following new members joined: Adolfo Martinez Palomo, Emeritus Investigator of the Center for Research and Advanced Studies of the IPN; Dr. Ruben Lisker Yourkowitzky, Director of Research of the National Institute of Medical Sciences and Nutrition Salvador Zubiran; Dr. Misael Uribe Esquivel, Chief of the Department of Gastroenterology of the National Institute of Medical Sciences and Nutrition Salvador Zubiran; and Dr. Teresita Corona Vazquez, General Director of the National Institute of Neurology and Neurosurgery Manuel Velasco Suarez. Later, in July 2009, Dr. Jose Narro Robles, Rector of UNAM, was invited to represent the educational sector as a substitute for Dr. Fernando Bilbao.

It is worth mentioning that according to Article 16 of the cited law, this Governing Board had the following non-delegable duties conferred by the Federal Law of Public Enterprises: (1) approve the distribution of the definitive annual budget of the entity and the investment programming, according to the total amount authorized by its budget; (2) approve budget adjustments for its programs, which does not imply changes in total authorized amount, investment resources, projects financed by external credit, or in the fulfillment of the institutional objectives and goals; (3) establish guidelines for the application of self-generated resources; (4) authorize the use of spaces in the areas and facilities of the Institute that are not for hospital use; (5) approve and modify the basic structure of the institution according to the total budget authorized for personal services; (6) establish a system for continuing professional education for the personnel of the Institute; (7) determine the rules and the percentages of additional salary for the personnel who participate in extramurally- funded research projects, and the royalties that result from the application or exploitation of industrial and intellectual property rights that are derived from projects performed by the Institute; and (8) evaluate and approve candidates for executive posts presented by the General Director. In parallel, according to Article 17, the Governing Board of the Institute carried out ordinary sessions twice each year in addition to the extraordinary sessions proposed by its Chair (Jiménez-Sánchez, 2009).

Based upon the above, the Governing Board of INMEGEN chose Dr. Gerardo Jimenez-Sanchez as the founding General Director of the Institute. He had served as Director of the Consortium for the Institute of Genomic Medicine. In addition, the 2004–2009 Work Program for the National Institute of Genomic Medicine (Jiménez-Sánchez, 2004a) was prepared by Dr. Jiménez-Sánchez as part of the selection process for INMEGEN's General Director.

THE BOARD OF TRUSTEES

The Board of Trustees was established according to Article 21 of the Law of the National Institutes of Health. According to Article 23 of the Law, this board comprised a president, a secretary, a treasurer, and the members, all of whom were recognized for their reputations, were part of the social and private sector or of the community in general, and who had a dedication to service. The operations of the Board of Trustees and the tenure of its members in their posts were established by corresponding internal rules of operation. It is worth mentioning that the posts of the members were honorific, and they did not receive remuneration, emolument, or compensation, according to Article 24 of the law mentioned above.

The Board of Trustees of INMEGEN assisted the Governing Board and the General Director through the development of the following functions, according to Article 25 of the cited law: (1) support the activities of the Institutes and formulate suggestions aimed at improving performance; (2) contribute to the procurement of resources that promote the fulfillment of the objectives of the Institutes; and (3) complete other functions indicated by the Governing Board.

Within this legal framework, in the first ordinary session of the Governing Board celebrated on March 30, 2005, the integration of the Board of Trustees of INMEGEN was authorized, according to the current regulations; the Board of Trustees was included as an advisory and consulting body, which had the goal of supporting the work program of INMEGEN, mainly by encouraging excellence in scientific research, by training highly specialized human resources, and by facilitating the link with the business sector that will allow the medical applications derived from genomic medicine research to be translated into goods and services that contribute to the healthcare of Mexicans.

In this context, in August 2004, the Secretary of Health, Dr. Julio Frenk Mora, president of the Governing Board of INMEGEN, invited Mr. Carlos Eduardo Represas de Almeida to join and preside over the Board of Trustees and to join the Governing Board of INMEGEN as a member. Mr. Represas has distinguished himself as a leading member of the Mexican Health Foundation, having served as Vice President of Finance of that organization, and as a member of the Board of Directors on various occasions. His performance as chair of the Board of Trustees was of great value in directing the activities of the organization to consistently benefit the institution and improve its performance.

The Board of Trustees of the Institute was composed of distinguished members of the business sector, whose work was fundamental in the support of INMEGEN's program of work. This collegial body was composed of the following founding members: Carlos Eduardo Represas

de Almeida (Chair), Jorge Arevalo Chavez (Secretary), Emilio Azcarraga Jean, Luis German Carcoba García, Henry S. Davis, Pierre Froidevaux Chavan, Marcos Martinez Gavica, Ernesto Rubio del Cueto, Jaime Serra Puche, and Nina Zambrano.

This board contributed in a creative way to add value to the development of the activities of INMEGEN, through a work program related to the one proposed by the General Director and approved by the Governing Board (Jiménez-Sánchez, 2004a). The board established a trust fund to receive funds on behalf of INMEGEN, and also performed various activities aimed at supporting the development of the Institute. Among these, there was the creation of an informative video on INMEGEN and the establishment of strategic partnerships, for example with Nestlé, a company headquartered in Switzerland that develops scientific research of the highest level, including studies related to genomics and proteomics. This relationship led to the establishment of two scholarships and a Nestlé Chair of Nutrigenomics to promote the development of this field in Latin America.

ALIGNMENT WITH FEDERAL PROGRAMS

The creation and operation of INMEGEN contributed to the development of one of the areas of interest indicated by the National Health Program of 2001–2006 (PRONASA); the creation of INMEGEN was considered to be within the scope of the tenth strategy: "Strengthen investment in human resources, research and infrastructure in health." In particular, this project was related to the objectives stated in action line 10.4 of the National Health Program: "Strengthen the research and development of health technology."

Because of the federal goals stated above, it is important to mention that INMEGEN's 2004–2009 Work Program was in the context of the 2001–2006 National Health Program, as well as the Health Research Action Program. In this context, the creation of INMEGEN responded to the challenge of improving health research quantitatively and qualitatively. In particular, the strategy was intended to generate scientific innovation and to make the development of genomic medicine in Mexico a reality through a multidisciplinary approach and a focus on sectorial collaboration and inter-sector liaison. With the creation of INMEGEN, the national health system and its public institutions were strengthened by the promise of research in genomic medicine and training of human resources.

Furthermore, to contribute to the objectives of the 2007–2012 National Health Program, INMEGEN aligned its efforts with the following strategies: fortify and integrate actions that promote health and prevent diseases; promote investment in systems, information technologies, and communications that improve the efficiency and the integration of the sector; strengthen research and training in the healthcare field; and support the ability to offer health services through the development of the necessary infrastructure and equipment.

THE 2004–2009 WORK PROGRAM FOR INMEGEN

The Governing Board of INMEGEN approved the Work Program for the period of 2004–2009 that was proposed by Dr. Gerardo Jiménez-Sánchez as part of the selection process of the General Director of the Institute. In this program, the mission, vision, and objectives of INMEGEN were established, which included priorities for the initial development of the Institute and which were grouped into nine strategies.

The mission of INMEGEN is "to contribute to the healthcare of Mexicans by developing excellent scientific research and providing high-level training for human resources, which will lead to the medical applications of genomic knowledge through an innovative culture, cutting-edge technology, and strategic alliances that adhere to universal ethical principles."

The vision of the Institute is "to be the undisputed leader in Latin America and one of the main research hubs worldwide for developing genomic medicine, undertaking scientific research, training human resources, encouraging technological innovation, and developing goods and services. The most important values to INMEGEN are excellence, honesty, creativity, responsibility, institutional pride, loyalty, and respect."

The Work Program included nine specific strategies with 44 action lines and 125 goals to achieve during the first five years of INMEGEN's existence. The strategies were as follows: (1) organizational design: "the INMEGEN system"; (2) high-level scientific research in genomic medicine; (3) excellence of training and teaching in genomic medicine; (4) cutting-edge genomic technology applied to medicine; (5) establishment of the initial infrastructure; (6) development of strategic alliances for the integral development of genomic medicine; (7) translation of scientific knowledge into health goods and services; (8) compliance with the ethical, legal, and social frameworks of genomic medicine; and (9) administration of the research and teaching services.

Currently, INMEGEN (www.inmegen.gob.mx) is one of the twelve NIHs of Mexico. INMEGEN constitutes the cornerstone of the Mexican strategy to develop a national program in genomic medicine. The organization is designed to grow as an autonomous institution, linked to the educational and health sectors across the entire country. Its main funding source comes from federal fiscal funds, although it also receives support in the form of donations, both national and international. During its

first three years of operation, the Mexican government assigned more than 125 million dollars for its initial operations and infrastructure. This included modern facilities located in Mexico City that consisted of high-technology genomic units for sequencing, genotyping and expression analysis, supercomputers, proteomics, and biomarker discovery laboratories. In addition, a research center for the ethical, legal, and social implications of genomic medicine was established, along with a center for the incubation of businesses and technology transfer. Very soon, INMEGEN will open its new 60,000m^2 facilities on the NIHs campus, in the south of Mexico City (Figure 99-1).

The scientific agenda of INMEGEN includes the understanding of the genomic structure of the Mexican population and the study of its most complex health problems, which include diabetes, obesity, cardiovascular diseases, infections, autoimmune diseases, macular degeneration associated with age, and tumors of the thyroid, breast, prostate, and blood. The majority of the projects involve the identification of genes associated with common disease risk and the biomarkers for their early diagnosis and prognosis. Furthermore, pharmacogenomics is an important part of the scientific agenda, given that strategies are being proposed to create more effective and less toxic medicines for the Mexican population (Seguin et al., 2007).

MAIN ACHIEVEMENTS OF INMEGEN DURING 2004-2009

During its first five years of operation, INMEGEN laid the foundation necessary to develop a national platform for genomic medicine in Mexico. The institute recruited a group of talented professionals who were dedicated to the construction of an innovative institution, and who shared an ambitious vision for the future. This vision was one of developing genomic medicine oriented to ease national health problems in the context of the programs established by the federal government.

The organizational design of INMEGEN was the product of careful planning aimed at developing a modern institution whose contributions would not only have a direct impact on public health, but would also be governed by ethical guidelines that guarantee adherence to universal ethical principles. Thus, the Institute established its Code of Ethics and Conduct, in addition to 34 internal normative manuals and instruments, which were incorporated into the daily activities of the personnel through more than ten seminars about organizational culture.

The main research areas considered in INMEGEN's 2004–2009 Work Program were population genetics, metabolic diseases (obesity and diabetes mellitus), cardiovascular diseases, cancer, infectious diseases, and pharmacogenomics. Over time, scientific progress generated the opportunity to broaden these areas of research. So, beginning in 2007, new areas of research were added, including medical proteomics, functional genomics for cancer, nutrigenomics, and genomic eye diseases. Currently, INMEGEN carries out genome-wide association studies (GWAS) on age-related macular degeneration, obesity, systemic lupus erythematosus, and cardiovascular diseases. INMEGEN also analyzes the expression of leukemias, sarcomas, and breast (Hidalgo-Miranda & Jiménez-Sánchez, 2009), lung, thyroid, and prostate cancers both in humans and in experimental mouse models

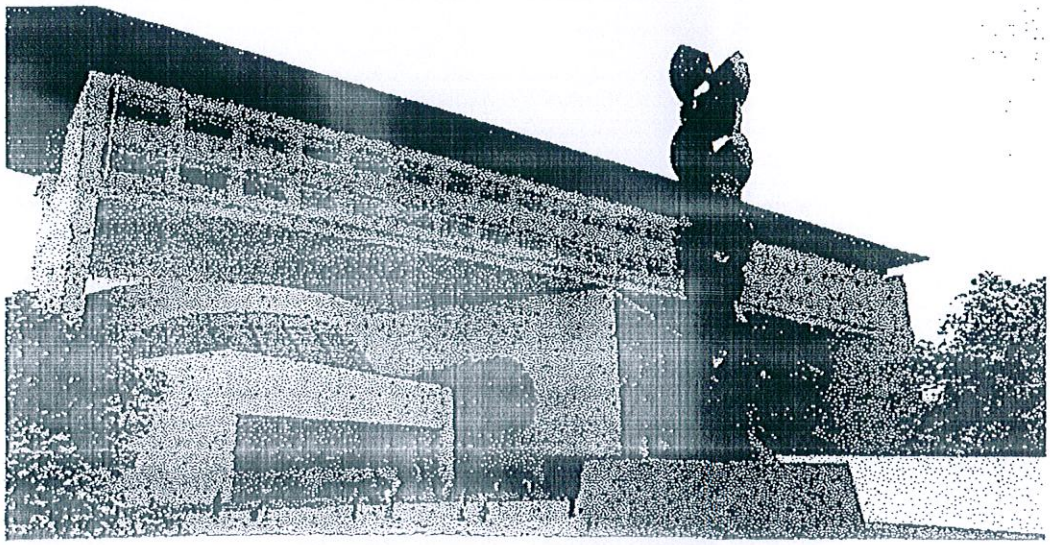

Figure 99-1 The National Institute of Genomic Medicine in Mexico City. Located in the Campus of the National Institutes of Health.

(Mendoza-Villanueva et al, 2008). In the area of medical proteomics, INMEGEN is working to discover biomarkers for melanoma, lung, and breast cancer through an analysis of the proteomic profiles of blood, saliva, and other body fluids.

ACADEMIC ACTIVITIES AND TECHNOLOGICAL INFRASTRUCTURE

The selection and recruitment process for the academic personnel of INMEGEN ensures that each member of the team can contribute to specific areas of medical genomics. By August of 2009, INMEGEN had recruited 31 researchers organized in nine laboratories, each of them with cutting-edge technology. They conducted 42 scientific research projects (Table 99-1), 11 of which received external financing. The results included over 55 peer-reviewed publications, training of human resources, and the first two National Congresses of Genomic Medicine in Latin America. In addition, a laboratory of genomic diagnosis was established to develop genomic services, initially those related to pharmacogenomics of oral anticoagulants (Acenocoumarol and Warfarin) and the antiretroviral Abacavir.

TABLE 99-1 SELECTION OF RESEARCH PROJECTS DEVELOPED AT INMEGEN, 2004-2009.

1. Genomic diversity of Mexican populations.
2. GWAS study for essential hypertension in the Mexican population.
3. Genomic structure of the angiotensinogen gene.
4. Genomic variability associated with age-related macular degeneration.
5. Analysis of proteomic profiles in the saliva of smokers with lung adenocarcinoma.
6. Resequencing analysis of genes related to drug metabolism.
7. Pharmacogenomics of Acenocoumarol and Warfarin.
8. Pharmacogenomics of Abacavir.
9. Analysis of maternal and paternal lineages with mitochondrial markers and of the Y-chromosome in the Mestizo population.
10. Genomic risk analysis of morbid obesity in the Mexican population.
11. The regulatory role of *CEMP1* during the process of in vitro cementogenesis/osteogenesis.
12. Identification of early biomarkers for diabetic nephropathy.
13. Genomic basis of autoimmune diseases among the Mexican population.
14. Identification of polymorphisms among genes associated with asthma.
15. Genetic risk factors for obesity and metabolic syndrome.
16. Allelic variants associated with the oxidative stress response in health and disease.
17. Genetic association with susceptibility to asthma and treatment response.
18. The Nrf2-Keap1 signaling pathway in the susceptibility to myeloid leukemia.
19. Early molecular biomarkers of liver cancer.
20. Global expression analysis in a three-dimensional culture model in different types of tumors.
21. Regulation of the expression of Sinac/DIABLO.
22. Sequence analysis of the amyloidogenesis of immunoglobulin light chains.
23. Proteomic biomarkers associated with melanoma.
24. Proteomic analysis of breast cancer and identification of cancer biomarkers.
25. Proteomic profiling in gastric cancer.
26. Ethical, legal, and social implications of genomic medicine.
27. Pan-American Initiative in Bioethics.

The high-technology units established at INMEGEN focused on supporting specific research and teaching programs of the Institute and other affiliated institutions. These units included the genomic sequencing and polymorphism analysis unit, powered by Affymetrix, Illumina, and Applied BioSystems platforms, the genomic expression unit, the medical proteomics and biomarker identification unit, and the validation area, which has the capacity to use tissue arrays for the simultaneous and comparative analysis of proteins. All of these units have access to the most advanced technology in genomic medicine.

The high-technology units were connected to a supercomputer and an information technology system, which had a capacity of over 2.2 million operations per second. This was done for the purpose of meeting the analysis requirements of the research projects. Additionally, a storage capacity of 22 terabytes was implemented, which was essential for storing the large quantity of genomic data generated by the various research projects. This infrastructure included computer and telecommunication networks to support the projects of both local scientists and others located in various institutions around the world.

Training of human resources increased rapidly, as various courses were formally integrated into undergraduate and graduate programs. Over 20 courses were offered during this period, attended by 455 students from across the country. Furthermore, more than 42 graduate students from various institutions of higher education carried out their scientific research projects in the laboratories of INMEGEN, obtaining their respective degrees.

INMEGEN hosted more than 130 academic events of the highest level and has been honored with the participation of distinguished international speakers. These events included the first and second National Congresses of Genomic Medicine. The participation of the medical, academic, and scientific community of Mexico and other parts of the world was overwhelming, with more than 20,700 individuals participating.

The initial facilities of INMEGEN included a Center for Information and Documentation (CID), which specialized in the different areas of genomic medicine. The CID collection contains more than 1100 books and subscriptions to 167 specialized scientific journals. Moreover, the innovative culture of the Institute led to the establishment of an electronic system to provide more than ten different electronic services to both the personnel of INMEGEN and interested parties in other geographic locations.

Since its creation, one of the priorities of INMEGEN has been the development of scientific, academic, and business interactions with various organizations in Mexico and abroad. During 2005–2009, 48 cooperation agreements were signed (Jiménez-Sánchez, 2009). Such agreements have contributed to the development of a national platform of genomic medicine, thus creating synergies that will assure permanent world-class competitiveness.

The strengthening of the links with the academic sector continued in 2006, through academic and scientific collaboration with the University of Guanajuato, the Autonomous University of Tamaulipas, the Autonomous University of the State of Morelos, the National Council of Science and Technology, and the College of Postgraduates. In 2007, INMEGEN's interaction with the Institute of Anthropological Research of UNAM led to the project, "Lineages of mitochondrial DNA and of the chromosome in the Mestizo and indigenous Mexican population."

Important alliances were established with the departments of health in various states of Mexico, an activity that began at the end of 2004 through an interaction with the state of Zacatecas, which established a program for visits and rotations for students. Another collaboration with the state of Yucatan led to a pilot project to study cardiovascular diseases in the Mestizo-Mayan population.

In 2005 and 2006, collaboration agreements were formalized with the states of Yucatan, Veracruz, Sonora, Guerrero, Zacatecas, Guanajuato, and Tamaulipas. These states participated in the project to develop a genomic map of Mexican populations. They also stimulated information exchange related to scientific progress in genomic medicine, academic activities, technological services, and publications, as well as training of human resources and development of research projects.

The link with the industrial sector became an ideal space to explore nutrigenomics, one of the areas with the greatest potential in the coming years. During the second semester of 2006, INMEGEN's interaction with Nestlé, S.A. de C.V. began. The following year, the relationship was formalized with the signing of a collaboration agreement for the establishment of the "Nestlé Chair of Nutrigenomics" and the "Nestlé Fellowships in Nutrigenomics." The purpose of these positions was to support scientific research and training of human resources in nutrigenomics at INMEGEN. The positions also served to foster new ideas that could contribute to improve healthcare of the Mexican population.

The inauguration of the Nestlé Chair in Nutrigenomics was planned for a period of three years, with the goal of recruiting a senior researcher capable of conducting research on fundamental topics related to improving the health status of Mexicans through nutrition and genomics. Furthermore, two masters, doctoral, or postdoctoral positions were created under the name, "Nestlé Fellowship in Nutrigenomics." These positions were designed to encourage young Mexicans to develop proficiency in the area of nutrigenomics.

The selection process for the awardees of those fellowships began with the publication of a call for applications in the last quarter of 2007; three applications for study in nutrigenomics and two applications for the position of Chair of Nutrigenomics were received. By 2008, a review process was established by an ad-hoc group. Currently, Dr. Elizabeth Tejero holds the Chair in nutrigenomics.

The links with foreign institutions were of great importance, and the first contacts with organizations outside of Mexico began in 2004. These international organizations included the National Institutes of Health of France (INSERM), the National Institutes of Health in the United States, Johns Hopkins University, and the Broad Institute of Harvard University and the Massachusetts Institute of Technology. Vanderbilt University had an important role in developing collaborative efforts in the areas of ethical, legal, and social implications of genomic medicine, as well as in the areas of bioinformatics and pharmacogenomics.

In 2005, strategic alliances were established with the Translational Genomics Research Institute (TGen), under the direction of Dr. Jeff Trent, which turned into specific projects of academic and scientific collaboration. Furthermore, beginning on December 20, 2005, INMEGEN joined the "Public Population Projects in Genomics" (P3G) under the leadership of Professor Bartha Knoppers, a nonprofit international consortium with the main objective of promoting collaboration among researchers in population genomics through the creation of a public, open, accessible and transparent database.

In 2006, a collaborative agreement was established with the Interdisciplinary Center on Bioethics of the Pan-American Health Organization/World Health Organization for the purpose of training human resources, developing collaborative research projects on ethical aspects of genomic medicine, and exchange of information. Several publications resulted from this effort, particularly in the area of ethical, legal, and social aspects of genomic medicine.

In 2007, an academic and scientific collaboration agreement was signed with the Genome Institute of Singapore, represented by Dr. Edison T. Liu, Executive Director of the Institute. The purpose of this agreement was to strengthen and broaden cooperation efforts to establish research programs and other collaborative projects. Through this link, both institutions developed research activities in genomics, pharmacogenomics, educational opportunities, and ethical, legal, and social matters related to genomic medicine.

During the same year, INMEGEN's international collaborations were particularly productive. A collaboration with the United Nations University of Biotechnology for Latin America and the Caribbean was established. This agreement included the granting of funds to INMEGEN for the design and delivery of the course "Ethical, Legal

and Social Matters in Genomic Medicine," which was the first of its kind in Latin America. Similarly, as a result of the agreement between the *Centre de Recherche en Droit Public* of the University of Montreal and INMEGEN, the HumGen Spanish portal (http://www.humgen.umontreal.ca/int/partenaires.cfm?&lang=3) was launched. This portal serves as a Spanish-language tool to explore the ethical, legal, and social aspects of genomic medicine. It could also serve as a communication bridge between Canada and Latin America on legal and social matters related to genomic medicine.

In addition, INMEGEN organized an "International Meeting of Leaders in Genomic Medicine: Emerging Regulatory Aspects" in collaboration with the Drug Information Association (DIA). The event had attendees and speakers from Mexico, Latin America, the United States, Canada, Switzerland, India, Singapore, Belgium, and Japan, among other countries.

INMEGEN created a Center for Research on the Ethical, Legal, and Social Aspects of Genomic Medicine, which brought together researchers from different institutions who together developed scientific studies on new topics in this field. The Intellectual Property Program was also developed, and a business incubator called ProGen was established (http://progen.inmegen.gob.mx). The work of ProGen was tightly linked to INMEGEN research projects and also included matters of copyright, associated with multiple brands and characters in comic books elaborated by INMEGEN.

The National Institute of Genomic Medicine has played a fundamental role in the dissemination of information about genomic medicine, and it has also earned national and international recognition. The methods used to disseminate information turned out to be very effective. Among them, the design of three widely distributed comics on genomic medicine stand out, as well as the design of a bilingual web portal that has become a key site to spread genomic medicine information. Over 53 million successful consultations were made through INMEGEN's portal between 2004 and 2009, and more than 45 million documents were downloaded by users from 140 countries.

In addition to INMEGEN's initial facilities, the construction of its permanent headquarters is now 80% complete (Figure 99-1). This new facility will provide a 17-fold increase in space and technological capacities that will be essential for the next phase of Mexico's construction of a national platform of genomic medicine (Hardy et al., 2008).

The results from INMEGEN's first administration were released to the public in 2009 in a four-volume report entitled: "Report on the 2004–2009 Activities of the National Institute of Genomic Medicine" (Jiménez-Sánchez, 2009). This documents reports the full implementation of INMEGEN's Work Program and was approved by the Governing Board of the Institute (Jiménez-Sánchez, 2004a).

THE MEXICAN GENOME DIVERSITY PROJECT

The characterization of the genetic structure in the Mexican population was one of the strategic areas of research at INMEGEN. Such information will be an essential tool for the development of genomic medicine for the Mexican population. The development of this line of research is of special relevance in the context of the initial phases of the International HapMap Project, which did not include any Latin American populations (HapMap, 2005).

Mexican Mestizos constitute a recently admixed population, mostly composed of European, Amerindian, and African lineages. The genetic heterogeneity of Mexicans is derived from a range of different demographic dynamics in geographically distinct regions. The first stage of the project included the genotyping of 110,356 SNPs from 300 unrelated individuals who identified themselves as Mestizos and originated from six geographically distant regions of Mexico (Silva-Zolezzi et al., 2009). Next, the genetic diversity, the patterns of linkage disequilibrium (LD), and the extent of common haplotypes were determined using data from these individuals along with information from the HapMap. The initial results of the project indicated that, even when there are regional genetic differences among Mexican subpopulations, these groups are sufficiently similar to be analyzed as one single group. However, the results of this study provided evidence of the population structure of Mexicans that should be considered when designing and analyzing genomic association to human disease. The initial analysis used a set of 2824 informative markers for ancestry derived from three populations of the HapMap. The analysis indicated that there are different proportions of mixes among Mestizos, and it showed a fourth ancestral component that is present in different proportions and that correspond to the Amerindian contribution (Silva-Zolezzi et al., 2009). The results of this project indicate that a haplotype map of Mexican Mestizos would improve the selection of tag-SNPs and make it possible to better capture common genetic variations in studies on the associations of diseases common among the Mexican population. This is the first genome-wide genotyping effort of a recently admixed Latin American population that is available to the general public (http://diversity.inmegen.gob.mx).

To generate a more comprehensive description of the common genetic variations and to adequately describe the genomic structure of these populations, the SNP density will be increased to approximately 1.5 million. In addition, the ensuing analysis will include Amerindian populations. The results derived from these efforts will provide the foundation for translating knowledge of the genetic structure of the Mexican population into a better understanding of common complex diseases. Additional studies on the genomic structure will include the systematic

analysis of copy-number variations in the Mestizo and Amerindian populations.

The initial results of this project suggest that the genetic differences among Mestizos of various regions of Mexico are mainly due to differences in the ancestral contributions of European and Amerindian populations. Comparative analysis of the proportion of these contributions among the participating states demonstrated important differences among some of them. In the majority of the analysis, the samples of the central regions had a higher similarity to indigenous groups, while the samples from the northern regions were more similar to European populations. These findings are supported by the current and historical Amerindian population densities in these regions (Silva-Zolezzi et al., 2009).

An analysis of the shared alleles and haplotypes was performed to evaluate the degree of genetic variability that is shared among samples of Mexican Mestizos and the populations of the international HapMap project (Europeans, Africans, Chinese, and Japanese). The results indicated that Mexicans share 64% of the common haplotypes (present in more than 5% of the samples) with Africans, 75% with the population of Asia, and 80% with the populations of northern Europe. When the information is combined with the four populations from HapMap, the percentage of shared haplotypes rose to 96%. These results indicate that there is a percentage of genetic variability present only among Mexicans, and that this variability is derived from the Amerindian component of the Mestizo population. On the other hand, the results of the comparison among Mestizo populations indicate that a specific haplotype map for them would allow a better selection and would reduce the number of genetic markers necessary for association studies of complex diseases, making these studies more efficient and less expensive.

The analysis of private alleles was performed for the purpose of identifying genetic variants exclusive to Mexican populations that had a frequency greater than 5%, and that were absent from the populations examined in the initial phases of the HapMap Project (African, European, and Asian). Eighty-nine markers were identified, only in some of the Mexican populations analyzed. In addition, 86 of these markers were also found in the samples of the Zapoteca population, which indicates their Amerindian origin (Silva-Zolezzi et al., 2009).

The initial results of the project demonstrated the feasibility of generating a public genomic database of the Mexican population and a catalog of the most common variants (http://diversity.inmegen.gob.mx). This public resource will become an important source of information for the design of genomic studies aimed at finding genes associated with common diseases, not only in Mexico but also in many parts of Latin America.

The Mexican Genome Diversity Project does not include direct medical applications, but rather was designed as a public tool that will lay the foundation for clinical studies on genomic medicine that will be performed by researchers across the country and in other parts of the world. In addition, it will allow new areas of research to be explored that will help identify genetic variations associated with a predisposition to common diseases, and how individuals with those genes respond to medications. This project was funded with public resources assigned to INMEGEN, and developed with important donations from the Mexican Health Foundation and the Gonzalo Rio Arronte Foundation.

The Mexican Genome Diversity Project has represented important progress for genomic sciences in Mexico. In addition, it has stimulated the recruitment of a critical mass of researchers in various disciplines. Furthermore, the technological infrastructure created by this project has become a valuable asset. These accomplished milestones, combined with our highly qualified scientists and the strategic liaisons established by INMEGEN, will contribute to develop a robust national platform in genomic medicine.

CURRENT AND FUTURE CHALLENGES

Mexico has invested significant efforts and resources to develop genomic medicine as a means to improve healthcare for the Mexican population. The results obtained in the first ten years of work have exceeded anyone's expectations in establishing the foundations of a mid-term national project.

Developing genomic medicine in Mexico to the point where it can perform at a leadership level and truly improve the healthcare of the Mexican population requires more than just solid scientific work. This ambitious goal will demand several cultural changes, including serious team efforts from medical clinics and scholars of the basic sciences, and a focus on common objectives. These collaborative efforts will be needed to address national health problems such as diabetes mellitus or breast cancer. It is likely that disarticulated projects will not be as successful with problems of this magnitude. It is important, therefore, that the Mexican model fosters a real component of innovation. In general, it will be the younger generations that will better deal with these challenges.

There are other challenges, including the need for new financing mechanisms, new incentives to innovate, and new ways to register intellectual property. The current model dominating scientific research has a significant impact on the scientists' income and creates a legitimate urgency to publish results. Although it has been proven that such systems increase the number and quality of scientific publications, they do not stimulate innovation in areas in which the risk of failure is significantly greater. In those areas, results are usually published after registration of the intellectual property. In recent years, the Mexican government has established programs to stimulate innovation

and to generate new business models based on scientific knowledge. These programs, located within CONACYT, reflect the awareness within the federal government of the strategic importance of innovation for economic growth. INMEGEN has established a synergy with CONACYT and the Mexican Institute of Industrial Property (www.impi.org.mx). The goal of this synergy is to develop mechanisms to incorporate innovation as a part of the local community, particularly in genomic medicine. Although there may be a number of genomic projects that will be developed by Mexican scientists alone, many genomic projects are generally the result of international collaborations, whereby Mexican scientists send samples to countries that have a more solid infrastructure. The goal of this collaboration is to combine forces and accelerate their research (Table 99-2A & 99-2B).

Even though international collaboration is desirable and encouraged, as the Mexican groups increase their critical scientific and technological mass, the collaborations tend toward a more equitable level of contribution, and groups are increasingly embarking on more independent research. Even though training programs in genomic medicine are recent, the number of applicants has significantly increased from dozens to hundreds at INMEGEN and the LCG. This clearly indicates that there is an opportunity to host new training programs in genomic sciences that respond to the needs of the current generations and contribute to the development of genomic medicine in Mexico. Unfortunately, "brain drain" continues to be a significant problem, mainly because of the lack of competitive salaries, the appropriate infrastructure, and funding opportunities for research throughout the country. The challenge of genomic medicine includes the recruitment and repatriation of qualified professionals, and the development of means to retain them for long enough to successfully develop their scientific contributions. In recent years, this situation has improved thanks to better infrastructure and consistent research programs that attract young researchers and bring them back to Mexico. CONACYT is facing this challenge by implementing a repatriation program, and a program aimed at retaining young scientists in Mexico. As a result, between 2001 and 2005, a total of 845 scientists were repatriated, some of which joined our genomic medicine program.

The limitations of sustained funding represent a challenge for science worldwide. Mexico's investment in research and development (R&D) is the lowest among member states of the Organization for Economic Cooperation and Development (OECD) and is equivalent to approximately a seventh of the average funding level of a member state of the OECD. It is significantly lower

TABLE 99-2A COLLABORATIVE AGREEMENTS SIGNED WITH INTERNATIONAL INSTITUTIONS 2004-2009

1. Translational Research Institute, Phoenix, AZ, USA
2. Bioethics Unit of the Pan American Health Organization – WHO.
3. Vanderbilt University, USA
4. McGill University, Genome Quebec, Canada
5. The State University of New York (SUNY), USA
6. United Nations University, UN
7. Genome Institute of Singapore, Singapore
8. Alcon Laboratories, USA
9. Centre de Recherche en Droit Public, Université de Montreal, Canada
10. Drug Information Association (DIA) USA
11. Foundation for the Popularization of Science and Technology and Institute of Science and Technology, Salamanca University, Spain

TABLE 99-2B COLLABORATIVE AGREEMENTS SIGNED WITH INSTITUTIONS FROM MEXICO 2004-2009

1. Yucatan Health Services; Autonomous University of Yucatan and FUNSALUD "Peninsular Chapter."
2. Zacatecas Health Services and Autonomous University of Zacatecas.
3. National Council of Science and Technology, MUO.
4. Sonora Health Services, the Autonomous University of Sonora and FUNSALUD.
5. Veracruz State Government and Veracruz University.
6. Ministry of Health of the State of Guerrero and Autonomous University of Guerrero.
7. National Institute of Public Health.
8. The State's Employees Social Security and Social Services Institute (ISSSTE).
9. National Institute of Public Health, Specific Agreement.
10. Guanajuato Health Services and Autonomous University of Guanajuato State.
11. Autonomous University of Morelos State.
12. Mexican Association of Human Genetics, MUO.
13. Mexican Association of Human Genetics, Specific Agreement.
14. Mexican Institute of Industrial Property.
15. "Gonzalo Río Arronte" Foundation.
16. Mexican Health Foundation, MUO.
17. Post-Graduate College (Agricultural sciences).
18. National Council of Science and Technology, HR Specific Agreement.
19. Tamaulipas Health Services and Autonomous University of Tamaulipas.
20. National Commission on Social Health Protection.
21. NESTLÉ, Mexico, MUO.
22. Ministry of Health of the State of Oaxaca; Autonomous University of Oaxaca "Benito Juárez" and Southeast Regional University.
23. Ministry of Health, Health Services of the State of Durango and State University of Durango.
24. Institute of Ophthalmology Conde de Valenciana Foundation.
25. Ministry of Health of the State of Campeche and Autonomous University of Campeche.
26. PROA Group Laboratories.
27. Federal District Government.
28. National Institute of Psychiatry "Ramón de la Fuente Muñiz."
29. National Autonomous University of Mexico.
30. Mexican Foundation for Promoting Education for Timely Prevention of Breast Cancer.
31. Genomi-k Laboratories.
32. Institute for Anthropological Research.
33. University Teaching and Research (Tec Milenio University).
34. Children's Interactive Museum (Papalote).
35. Hospital Juarez of Mexico.
36. Medix Products.
37. Nestlé México S.A.

than that of other emerging economies, such as China (0.7%), India (0.8%), or Brazil (>0.8%; OECD, 2006; Hardy et al., 2008). For comparison with other countries in Latin America, in 2004, Mexico invested 0.41% of its GDP in R&D, while Argentina invested 0.44%, Chile invested 0.68% and Brazil invested 0.91%. This represents a significant challenge for the successful development of a nascent field such as genomic medicine in Mexico. The Mexican government has not yet committed itself to investing in this promising field, but it is essential that the R&D situation be improved among public institutions in Mexico and that the technological infrastructure be kept updated. To reduce reliance on the federal government, various initiatives have been explored that provide additional support for genomic medicine. These include competition for international funding opportunities and increased private sector participation in R&D. Currently, around 35% of the spending on science and technology research in Mexico comes from the industrial sector (OECD, 2006). Even though this percentage is one of the lowest among member countries of the OECD, strategic alliances are being established between the industrial sector and public research institutions for specific projects, including those related to genomic medicine.

There are other challenges that play an important role in the successful development of genomic medicine. Some of these challenges involve the importation of equipment and chemical reagents from other countries. This is particularly important for the generation of new genomic technologies, because acquiring them involves significant costs from customs duties and tariffs, which must be paid for by the research budget. In addition, the excessive regulations and lengthy customs procedures damage many pieces of equipment and cause unfortunate delays during research projects.

As genomic medicine develops in Mexico, the need for modern legislation addressing both the ethical and social implications of genomic medicine will also increase. Questions of discrimination, confidentiality, equality in access to medical services, and financial and labor implications, among others, will require regulations based on Mexican laws.

We have learned valuable lessons during the planning and implementation of this strategy (Seguin et al., 2008). Some of them may be useful for similar emerging economies interested in developing genomic medicine. Careful planning is needed, along with the participation of the main organizations in the fields of health and education. Scientific and philanthropic organizations must work together on an equal level, which requires solid leadership at the highest levels to coordinate all of the efforts. It is important to determine the degree of academic, scientific, financial, and political capacity available before trying to establish feasible goals. It is also useful to take advantage of the local experiences of the past cases of both success and failure to design a more effective effort, to focus on ambitious but realistic goals, and to define specific challenges. These goals should include the development of programs for training, the development of infrastructure, and the selection of alliances. In addition, it is desirable that scientific research includes a component to translate knowledge into products and services, so that the programs are more attractive for public–private investment and contribute to the knowledge economy. Despite the large number of challenges ahead, Mexico has successfully begun to develop an expertise in genomic medicine. There is still much to do, but by carefully planning the commitments of the different actors in Mexican society, and by forming solid alliances, genomic medicine in Mexico will flourish and benefit the population through improved healthcare. Additionally, the country's scientific work, infrastructure, and commitment to innovation will have an effect throughout Latin America and will allow Mexico to participate in the worldwide transition toward a knowledge-based economy.

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¿Por qué un fármaco funciona en unas personas y no en otras? ¿es posible modificar los genes defectuosos sin afectar a los sanos?...Algunas de estas preguntas se están empezando a contestar en estos momentos gracias a los avances en la farmacogenómica, un área de investigación innovadora y de reciente desarrollo, y que en nuestro país tiene un referente mundial: el **Centro de Investigación Biomédica EuroEspes** (CIBE), situado en Bergondo, La Coruña, y dirigido por Ramón Cacabelos. En definitiva, se trata de aprovechar los recientes conocimientos que existen sobre el genoma humano para diseñar y desarrollar fármacos a medida del paciente, de sus necesidades y sus defectos genéticos.

"Las variaciones genéticas que existen entre las personas son una limitación fundamental para los tratamientos actuales que se utilizan en el manejo de las enfermedades del Sistema Nervioso Central. Cada persona reacciona de una forma diferente a un fármaco, de ahí la necesidad de introducir tratamientos a la carta", asegura Ramón Cacabelos. A su juicio, "la farmacogenómica servirá para optimizar el rendimiento de los fármacos, para dirigir el fármaco a la persona adecuada, para evitar efectos secundarios y para evitar costes, es decir, no trabajar con ensayo y error, sino dirigir el medicamento a la persona adecuada y a la patología adecuada".

Los progresos que se están logrando en este ámbito, a pesar de su corta vida, requieren un urgente cambio de mentalidad, tanto de las autoridades sanitarias como de la propia industria farmacéutica, un cambio de mentalidad que es fundamental para que la farmacogenómica evolucione. "Hay que pensar en la calidad de la atención, y eso pasa por ser más racionales y específicos en el uso de tratamientos. En estos momentos, la única manera de optimizar la respuesta terapéutica y de eliminar los efectos secundarios de los medicamentos es con la farmacogenómica", recuerda Ramón Cacabelos.

A pesar de la resistencia inicial ante todo lo nuevo, incide el Dr. Cacabelos, "la farmacogenómica acabará por imponerse en menos de 20 años. No hay alternativa; además, existe la ventaja adicional de que se puede aplicar conceptos farmacogenómicos a un antibiótico, a un antiinflamatorio, a un cardiotónico, a un fármaco para los ojos o a una crema tópica y, por supuesto, a los medicamentos del sistema nervioso. De hecho, se está utilizando ya en la esquizofrenia, en la depresión, en la enfermedad del Alzheimer. Esto demuestra que la farmacogenómica es una ruta inevitable con la cual, tarde o temprano, la industria y la Medicina se tienen que encontrar".

La ventaja de la farmacogenómica, en este sentido, es doble: por una parte, se centra en el diseño de medicamentos dirigidos a una enfermedad concreta que tiene un marcado carácter genético y, por otra parte, en el diseño de medicamentos que eviten toxicidades debido a un fallo metabólico.

Se trata de utilizar el conocimiento de genómica para desarrollar fármacos a los que responda un defecto genético en concreto y no otro. "Cuando hay una enfermedad genómica detrás, los medicamentos hay que dirigirlos hacia aquel gen que responde; así se evita utilizar fármacos a granel, se previenen una gran cantidad de efectos secundarios, y se soluciona el hecho de malgastar fármacos en un 30 ó 40% de los pacientes que no responden", asegura Ramón Cacabelos.

Gracias a los avances registrados en el conocimiento del genoma humano, se sabe que las enfermedades genéticas, fundamentalmente, son de dos tipos: las enfermedades mendelianas (las menos frecuentes), que se deben a un fallo genético puntual (si tienes la mutación, se te manifiesta la enfermedad y eso sigue las leyes de Mendel); por otra parte, están las denominadas enfermedades alogénicas y multifactoriales, que están asociadas a múltiples defectos distribuidos a lo largo del genoma humano y que, además, están influidas por el entorno (en esta categoría están el 80% de las patologías del sistema nervioso central). En EuroEspes, según apunta Ramón Cacabelos, "identificamos los grupos de genes que están asociados con la etiopatogenia de una enfermedad degenerativa neurológica o psiquiátrica concreta, y estudiamos cómo esos genes inciden en la enfermedad y el papel que tienen".

Con los conocimientos actuales, se sabe que la enfermedad de Alzheimer se asocia a más de 30 genes distinguidos a lo largo del genoma, donde unos están mutados y otros no. Pero es el resultado de la integración de todos esos genes lo que va a hacer que al final se manifieste o no. Cuantos más genes estén afectados, la enfermedad se manifiesta antes, tiene un curso mucho más rápido y responde peor al tratamiento. Cuantos menos genes estén afectados, la enfermedad aparece más tarde, su curso es mucho más lento y la respuesta terapéutica es mucho mejor. Entonces, lo que se hace con la genómica funcional es analizar cómo interactúan todos estos factores, mientras que con la farmacogenómica se consigue que un determinado fármaco actúe sobre el genoma afectado de ese paciente. "En este sentido, en EuroEspes, por una parte, hacemos el diagnóstico genómico integrando todos los genes posibles y, al mismo tiempo, investigamos la respuesta terapéutica asociada a ese colectivo genético concreto", destaca el Dr. Cacabelos.

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Farmacogenómica y la transición a un nuevo modelo farmacéutico: implicaciones económicas y de regulación

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INTRODUCCIÓN

En la última década los avances científicos han hecho posible diagnosticar y tratar un número creciente de enfermedades, en etapas más tempranas y con mayor precisión, para las que no existían respuestas clínicas efectivas. Estos avances han aumentado la capacidad de los médicos para individualizar tratamientos, maximizar la efectividad de las terapias farmacológicas y minimizar sus efectos adversos. Sin embargo, la traducción en hechos de las expectativas generadas en la descodificación del genoma no ha sido tan veloz como se esperaba, y la industria farmacéutica, los agentes reguladores, el colectivo médico y los pacientes se han visto en la necesidad de recalibrar esas expectativas.
Respecto a la industria, el agotamiento del modelo de blockbusters la ha llevado a plantear la transición hacia un nuevo modelo basado en la segmentación de pacientes y enfermedades (medicina estratificada), y cuyos instrumentos de vanguardia son las nuevas opciones tecnológicas aportadas por la Farmacogenómica. El objetivo es doble: por un lado, inyectar eficiencia en el proceso de descubrimiento, investigación y desarrollo de nuevos fármacos; por otro lado, introducir terapias farmacológicas, de alta efectividad y mínimos efectos adversos, dirigidas a subpoblaciones de pacientes discriminadas según la información farmacogenómica. En relación con las agencias reguladoras, se podría decir que se han visto "desbordadas" por la aplicación incipiente de los instrumentos farmacogenómicos, tanto en su vertiente clínica como en la vertiente de la investigación y el desarrollo. En efecto, las medidas

de regulación van por detrás de los avances en esa área y, aunque ya se han implementado algunas actuaciones relevantes (por ejemplo, la Critical Path Initiative por la Food and Drug Administration [FDA]), queda mucho por hacer.
Se argumenta que las razones principales de esas dos distintas velocidades podrían ser las siguientes: la aplicación de la Farmacogenómica no ha alcanzado todavía la madurez en términos de evidencia empírica sobre su impacto en salud pública; los reguladores y el colectivo médico no han ajustado sus conocimientos y comprensión de las potencialidades de la Farmacogenómica; un gran volumen de información generado por la Genómica y la Proteómica no se ha traducido en conocimiento; el cambio de modelo tiene unas implicaciones económicas que todavía están por ver; la introducción de las opciones farmacogenómicas requiere una profunda transformación de los sistemas sanitarios desde un enfoque médico-curativo a otro basado en la prevención y el diagnóstico; las implicaciones de la farmacogenómica en términos éticos y de equidad de acceso plantean serias dudas.
Las incógnitas señaladas tienen su máxima expresión en la actitud del colectivo médico que, sin formación actualizada y sin respaldo regulador, se muestra escéptico respecto a la utilidad clínica de la combinación de test diagnóstico y terapia asociada, aun en aquellos casos en que esa combinación ya ha demostrado plenamente sus bondades. Finalmente, los pacientes son los que manifiestan las mejores expectativas acerca del nuevo modelo, pero muestran preocupación sobre el uso de la información genómica y sobre el impacto que pudiera tener en términos de discriminación de coberturas sanitarias y de reembolso. Este trabajo pretende plantear las siguientes cuestiones y, en lo posible, ofrecer información y argumentos para encontrar respuestas: ¿cuáles son las preferencias de la industria farmacéutica respecto a las actuales opciones tecnológicas de la Farmacogenómica?, ¿qué implicaciones clínicas y económicas se derivan del nuevo modelo de medicina estratificada?, ¿qué criterios determinan la viabilidad del nuevo modelo y cuáles son sus obstáculos?, ¿qué impacto económico podría tener la medicina estratificada y qué parámetros podrían emplearse para evaluarlo?, ¿qué aspectos requieren cam-

bios en los sistemas de regulación para facilitar la adopción de los instrumentos farmacogenómicos?

SITUACIÓN ACTUAL DE LA FARMACOGENÓMICA

La incorporación de la Farmacogenómica en la práctica clínica tiene como potencialidades mejorar la eficacia y reducir la toxicidad al permitir la elección del "fármaco específico, para el paciente específico, en la enfermedad específica, con la dosis específica". Lo anterior representa un drástico cambio cultural en la práctica clínica que requiere la transición de un análisis de evidencia empírica basada en datos poblaciones a un análisis centrado en información individual para la elección de una terapia farmacológica. En la actualidad, es aventurado estimar el impacto, clínico y económico, de la aplicación de la Farmacogenómica, debido a que existe un número relativamente limitado de ejemplos prácticos, poca evidencia científica asociada a la práctica clínica actual y un uso restrictivo por parte de los proveedores asistenciales de los productos de la Farmacogenómica (incluso de aquellos con demostrada efectividad clínica).
Algunos de los éxitos pioneros de la Farmacogenómica, y de los más citados en la literatura científica, se refieren a Herceptin
®

(trastuzumab) para el abordaje del cáncer de mama precoz y metastásico; a la terapia farmacológica con tiopurina 6-mercaptopurina para tratar la leucemia linfoblástica aguda infantil; al anticoagulante warfarina para prevenir la formación de coágulos de sangre; a Glivec®
(imatinib), un inhibidor de la tirosín-cinasa para pacientes con leucemia mieloide crónica; y al anticancerígeno Camptosar®
(irinotecán). Aparte de estos éxitos indudables en cuanto a terapias farmacológicas asociadas a un biomarcador específico y de otros ejemplos actualmente en desarrollo, algunos sentencian que la promesa de la Farmacogenómica no ha sido satisfecha y que es preciso calibrar de nuevo las expectativas en el corto plazo (1).

Por una parte, se ha señalado que la investigación actual en Farmacogenómica debería reconducirse para obtener una mayor probabilidad de éxito e impacto clínico.

Esta afirmación se basa en tres premisas:

- La mayor parte de la investigación en Farmacogenómica se ha centrado hasta la fecha en el análisis de variaciones genéticas hereditarias. Sin embargo, en muchas condiciones clínicas la mayor carga de la enfermedad es originada por variaciones somáticas (no hereditarias), tales como los tumores cancerígenos.
- La mayor atención de la Farmacogenómica se centra actualmente en un número reducido de nuevos avances moleculares; y, a pesar de ello, los mayores beneficios potenciales de la Farmacogenómica se vislumbran en productos farmacológicos ya existentes.

En efecto, la mayoría de los efectos adversos, incluyendo aquellos originados por el perfil genético, surgen con fármacos ya consolidados en el mercado (2).

- Discrepancia de intereses entre la industria y los centros de investigación públicos. En efecto, las compañías farmacéuticas limitan la aplicación de la Farmacogenómica casi exclusivamente al proceso de investigación y desarrollo de nuevos fármacos. En este sentido, la Farmacogenómica no ha podido demostrar todavía sus potencialidades en los siguientes ámbitos: "rescate de fármacos" (productos retirados por sus efectos adversos o por su baja eficacia en un número reducido de pacientes); estrategias para ampliar el mercado de los fármacos existentes; análisis y seguimiento de productos en fase de poscomercialización; y empleo de datos de eficacia de fármacos ya consolidados. A su vez, el mundo académico centra su investigación farmacogenómica en mejorar la seguridad y la eficacia de productos que actualmente están en el mercado.

Por otra parte, y en este caso más relacionado con el entorno asociado a la farmacogenómica, se identifican una serie de obstáculos que actuarían en contra de su expansión. En primer lugar, la inexistencia de un desarrollo en paralelo de sistemas de financiación que priman el diagnóstico y la prevención por encima de la mera actividad asistencial curativa. En segundo lugar, sistemas de información segmentados que resultan inadecuados para los requerimientos de la investigación farmacogenómica.

En tercer lugar, una estructura de regulación obsoleta e incapaz de incentivar las potencialidades y de identificar los cuellos de botella asociados a este tipo de investigación. En cuarto lugar, programas de formación asistencial que todavía no se ajustan a las necesidades de la era genómica y proteómica. En consecuencia, el uso de los productos farmacogenómicos (por ejemplo, de biomarcadores o de la combinación de terapias que asocian fármacos y test diagnósticos) se encuentra limitado en el punto de

provisión. Finalmente, los avances en las aplicaciones farmacogenómicas plantean dudas (y resistencias) sobre posibles disparidades en el acceso a cuidados de salud y en la protección de información genética confidencial.

Principales opciones tecnológicas asociadas a la Farmacogenómica

Un número de opciones tecnológicas derivadas de la Farmacogenómica están al alcance de la industria farmacéutica. Sin embargo, algunas de estas opciones están recibiendo un interés y un volumen de inversión mayores dependiendo de factores tecnológicos, reguladores, comerciales y de predisposición por parte de los proveedores asistenciales.
Se pueden identificar cuatro ámbitos generales para la aplicación práctica de la Farmacogenómica:

- Investigación y desarrollo de nuevos fármacos.
- Mejoras en seguridad y eficacia de fármacos en desarrollo.

- Mejoras en seguridad y eficacia de fármacos comercializados.
- Estratificación de enfermedades.

En relación con el primer ámbito de aplicación, las compañías farmacéuticas están cada vez más interesadas en emplear técnicas y datos derivados de la Farmacogenómica para mejorar el proceso de descubrimiento de nuevos fármacos. Su aplicación es doble. Por una parte, una primera estrategia es identificar aquellos compuestos químicos que no muestren variabilidad significativa cuando se "dirigen" contra un objetivo (target) genómico específico. Los compuestos que superan el filtro reducen la probabilidad de ser retirados en etapas posteriores de las fases preclínica y clínica por razones de eficacia y/o seguridad. Por otra parte, una segunda estrategia es crear nuevos fármacos para subpoblaciones específicas con un perfil genómico similar y que respondan de forma positiva a la terapia. Esta estrategia aumenta la probabilidad de que el fármaco sea aprobado, a costa de serlo en un mercado más restrictivo.
Por lo que respecta al segundo ámbito, la Farmacogenómica puede dar origen a un diseño de ensayos clínicos más eficaces y a un "rescate" de compuestos químicos en las últimas etapas de desarrollo. En el diseño de ensayos clínicos, el empleo de biomarcadores en estudios preclínicos (o en fase I de desarrollo clínico) sirve de mecanismo de exclusión (inclusión) de subpoblaciones con un perfil genético específico con la finalidad de aumentar la probabilidad de éxito en términos de seguridad. Asimismo, en fases II y III la discriminación en función del genotipo puede aumentar la probabilidad de eficacia por dos vías: de manera prospectiva, centrando el análisis exclusivamente en subpoblaciones cuyo genotipo identifica a individuos de "respuesta positiva"; y, de manera retrospectiva, cuando se observa que el compuesto exhibe sólo un beneficio marginal en una población de pacientes, la estrategia es identificar a un subgrupo genómico en el que el nivel de eficacia es relativamente superior.
En cuanto al "rescate" de compuestos en desarrollo, la estrategia radicaría en recuperar en las últimas fases clínicas (fases II y III) aquellas entidades moleculares cuyos efectos adversos se concentran en un número reducido de pacientes. Este subgrupo de pacientes, identificados con un perfil genómico determinado, serían excluidos de posteriores ensayos clínicos. En este

sentido, la aprobación del fármaco se limitaría a subpoblaciones específicas y se ofrecería en combinación con un test diagnóstico. Similar procedimiento se aplicaría para aquellos compuestos que fracasan en fase III en cuanto a su eficacia en una población.
En lo que se refiere al impacto de la Farmacogenómica en fármacos ya comercializados, la fase IV de desarrollo clínico implica la exposición de un gran número de pacientes al fármaco en cuestión; la detección de efectos adversos normalmente ocurre en esta etapa. La recogida y almacenamiento de muestras de ADN de pacientes tratados en esta fase podría permitir el Screening farmacogenético y la identificación genética de factores de predisposición, con la finalidad de mejorar la ratio riesgo-beneficio de las terapias farmacológicas. La recogida prospectiva de muestras de ADN es una posibilidad en fase IV, pero podría ser inviable económicamente para la industria farmacéutica sin la creación de un entorno apropiado por parte de las autoridades sanitarias. Asimismo, el coste del empleo de biomarcadores para identificar y excluir pacientes que no responden al tratamiento en fase IV podría compensar ampliamente el coste de la prescripción de fármacos ineficaces para subgrupos de pacientes. Sin embargo, en este caso podría existir conflicto entre el interés comercial y el objetivo de salud pública asociado a la sobreutilización de fármacos.
Finalmente, el cuarto ámbito general de aplicación de la Farmacogenómica se refiere a la segmentación de pacientes (y de enfermedades) a través del empleo de biomarcadores para la identificación de subgrupos, previamente a la prescripción de terapias farmacológicas. Uno de los aspectos clave de esta estrategia radica en el grado de sensibilidad, especificidad, exactitud y utilidad clínica del test diagnóstico. Esta estrategia, con ejemplos ya consolidados en la práctica clínica habitual (biomarcador HER2 positivo, CYP450, TPMT testing , etc.), requiere una serie de ajustes y cambios en el entorno sanitario para su extensión efectiva, por ejemplo, aspectos reguladores referidos a la evaluación y aprobación combinada de fármaco y test diagnóstico.

Prioridades comerciales de la industria farmacéutica respecto a la Farmacogenómica

El interés de la industria farmacéutica en este campo de la investigación se traduce en que la mayor parte de las grandes compañías ya han desarrollado internamente programas de Farmacogenómica. El objetivo principal de este esfuerzo es reducir la ratio de fracaso –y, en consecuencia, los costes de los compuestos químicos en el proceso de investigación y desarrollo. Asimismo, la demostración de mayores niveles de eficacia a través de la discriminación de subgrupos de población asociados a una respuesta farmacológica positiva ha llevado a algunas compañías a desarrollar nuevos productos en combinación con test diagnósticos.
Los ejemplos de mayor éxito se refieren a la prescripción del anticuerpo monoclonal Herceptin®
(Genentech/Roche) para el cáncer de mama y de Erbitux®
(Imclone/Bristol-Myers Squibb) para el cáncer colorrectal.
La efectividad de ambas terapias está estrechamente asociada al resultado de sus respectivos kits diagnósticos.
De hecho, el fruto más obvio hasta la fecha de la investigación farmacogenómica se refleja en la producción de test diagnósticos (3).

La Tabla I muestra el posicionamiento de las compañías especializadas respecto a las oportunidades que

puede ofrecer la Farmacogenómica. Las conclusiones que se derivan son las siguientes:

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El mayor interés comercial se centra en las opciones farmacogenómicas aplicadas a la investigación y al desarrollo de nuevos fármacos. El 64% de las compañías implicadas en Farmacogenómica invierten en este ámbito; realizan un esfuerzo muy significativo para aumentar los niveles de seguridad (24 compañías) y eficacia (23 compañías) de productos farmacéuticos en desarrollo.

Tabla I.

Prioridades de la industria en cuanto a opciones tecnológicas de la Farmacogenómica

Productos	Número de compañías implicadas en Farmacogenómica
Desarrollo de nuevos fármacos	
Nuevos fármacos que funcionan en una población • Ensayos para variaciones genéticas 8 excluyendo a candidatos con un genotipo asociado asociadas a ADME a efectos adversos • Librerías de compuestos no metabolizados por genotipos específicos.	
Nuevos fármacos dirigidos exclusivamente a subgrupos • Ensayos para modelizar subtipos 9 genómicos específicos particulares de enfermedad Seguridad y eficacia de fármacos en desarrollo Seguridad en fases preclínicas • Ensayos para variaciones genéticas 24 asociadas a ADME	
• Test diagnósticos y chips para genotipos clínicos	
"Rescate" por el aumento de la seguridad en fases clínicas • Fármacos finalmente "recuperados" 6	
Aumento de la eficacia en "grupos de respuesta positiva" • Nuevos fármacos dirigidos a subpoblaciones 23 con un perfil genómico específico	
• Test diagnósticos y chips para genotipos clínicos	
"Rescate" por el aumento de la eficacia en fases clínicas • Fármacos finalmente "recuperados" 4	
Seguridad y eficacia de fármacos en comercialización	
Extensión del mercado para productos con efectos adversos •	
Ensayos y chips para	
CYP testing	
1	
• Test diagnósticos para identificar subgrupos de riesgo de efectos adversos Screening para detectar a pacientes propensos a efectos • Test diagnósticos para identificar 11 adversos subgrupos de riesgo de efectos adversos	
Monitorización de fármacos • Test diagnósticos para identificar 2	
subgrupos de riesgo de efectos adversos	
Screening para detectar a pacientes con "respuesta positiva" • Test diagnósticos para identificar 16	
subgrupos con mayor probabilidad de beneficiarse de la terapia farmacológica	
Uso de los datos de eficacia para el marketing y extensión • Test diagnósticos para identificar 3	
del periodo de patente subgrupos con mayor probabilidad de beneficiarse de la terapia farmacológica	
Segmentación de enfermedades	
Segmentación en subgrupos de enfermedades • Test diagnósticos para identificar 10 y agentes infecciosos	
subgrupos con mayor probabilidad de beneficiarse de la terapia farmacológica	

ADME: absorción, distribución, metabolismo y excreción.

Fuente: elaboración propia a partir de Hopkins et al. (3) y Martin et al. (21).

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Se identifican dos mercados prioritarios de interés comercial. El primero de ellos integra aquellos productos farmocogenómicos aplicados al desarrollo preclínico y clínico de nuevos fármacos. El segundo mercado se refiere a la producción de test diagnósticos para determinar

la prescripción de fármacos (principalmente ya comercializados) y para segmentar enfermedades y pacientes.

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Las empresas especializadas que desarrollan tecnologías para determinar la prescripción dirigen su interés mayoritariamente a la producción de test diagnósticos. La utilidad de estos test estriba, en primer lugar, en el objetivo de eficacia (16 compañías); seguidamente, en razones de seguridad (11 empresas); y, finalmente, en la segmentación de enfermedades (10 compañías).

La Tabla II muestra las principales áreas terapéuticas de aplicación de los test diagnósticos comercializados y en desarrollo. Se identifica el área del metabolismo (test dirigidos a enzimas que actúan en el metabolismo de los fármacos) como la de mayor atracción de esta opción tecnológica (17 test diagnósticos).

Comprender (y predecir) el metabolismo de los fármacos en el organismo es un aspecto crucial, ya que un metabolismo más lento de lo normal puede causar que el fármaco permanezca más tiempo del necesario en el cuerpo y en concentraciones que pueden ser perjudiciales para la salud. En cambio, un metabolismo demasiado rápido eliminaría el fármaco antes de que pudiera tener un efecto terapéutico positivo. El empleo de test diagnósticos con objeto de identificar el estatus CYP del paciente es una información de gran utilidad para el prescriptor a la hora de establecer la dosis correcta y efectiva de medicación. En general, los test aplicados en este ámbito tienen como consecuencia una segmentación de los pacientes, que siguen la misma terapia, pero son discriminados en virtud de la dosificación.

La segunda área terapéutica en la que más se aplican test diagnósticos se refiere a los fármacos anticancerígenos (12 test diagnósticos). El objetivo es identificar el perfil genético del tumor como medio para segmentar el cáncer en subtipos, según la respuesta a quimioterapias nuevas o que ya existen. En el caso del test Oncotype DX
®
, aplicado en terapias ya existentes como el tamoxifeno, su función consiste en segmentar a las pacientes con cáncer de mama según la probabilidad de recaída. En cuanto al test HER2 (por ejemplo, la inmunohistoquímica [IHQ] y la hibridación in situ fluorescente [FISH]), identifica a un subgrupo genético de pacientes con cáncer de mama cuyos tumores son susceptibles de obtener un alto grado de efectividad del anticuerpo monoclonal Herceptin(trastuzumab). Ambos test tienen el objetivo de hallar la terapia más efectiva para el paciente sobre la base de su perfil genómico. Por su parte, el objetivo del test UGT1A1 Molecular Assay es identificar a pacientes que presentan alto riesgo de sufrir efectos adversos derivados de la terapia anticancerígena con irinotecán para abordar el cáncer colorrectal.

Finalmente, la tercera área de mayor importancia relativa en la aplicación de test diagnósticos corresponde a los antivirales (ocho test diagnósticos). En este caso, los test no identifican las características genéticas del paciente, sino que más bien pretenden detectar la variedad viral que resulta ser resistente a fármacos específicos.

En particular, actualmente existen test diagnósticos en el mercado para identificar resistencia a las terapias VIH y hepatitis C. Sin embargo, tienen sus limitaciones, dado que la respuesta y la toxicidad son frecuentemente el resultado de interacciones complejas entre genes, factores ambientales, e incluso de un bajo cumplimiento terapéutico por parte del paciente.

Tabla II.

Test de diagnóstico farmacogenómico comercializados y en desarrollo

Tipo de test diagnóstico	Uso actual		En desarrollo		Todos los test
	EE. UU.	Unión Europea	Total	EE. UU.	Unión Europea	Total
Para el metabolismo de los fármacos	7	8	15	1	1	2	17

Para el abordaje del cáncer 3 3 6 3 3 6 12 (segmentación de enfermedades)
Para la resistencia de los fármacos 4 1 5 2 1 3 8 antivirales
Para otras condiciones 3 1 4 5 3 8 12
Total 17 13 30 11 8 19 49
Fuente: Martin
et al.
(21).

MEDICINA ESTRATIFICADA: TRANSICIÓN HACIA UN NUEVO MODELO ECONÓMICO

La necesidad de cambiar de modelo en el sector farmacéutico está ligada al cambio experimentado en la última década en la filosofía del proceso de investigación y a un ajuste más realista de las expectativas asociadas a la decodificación del genoma.
Hoy en día, el descubrimiento de un nuevo fármaco ya no se inicia con un potencial compuesto químico per se, sino más bien con una posible diana biológica.
Una vez que se ha identificado una diana, debe ser validada, es decir, testada para determinar su función y asegurar que es susceptible de ser modulada por un compuesto químico para producir un efecto terapéutico.
Los compuestos químicos que deben interactuar con la diana biológica pueden diseñarse a partir de cero o ser identificados a través del screening de colecciones de compuestos (library compounds)
. Las entidades moleculares seleccionadas deben pasar un proceso de optimización que implica: variaciones de su actividad, mejoras en sus propiedades farmacocinéticas y evaluación de la probabilidad de causar efectos adversos. Los compuestos que consiguen superar esas barreras son elegibles para entrar en las etapas preclínica y clínica. Una vez superadas estas últimas, se someten a la aprobación, a la producción y a la comercialización.
Este largo y complejo proceso de descubrimiento, investigación y desarrollo de un nuevo fármaco se ve afectado en los últimos años por una serie de características que apuntan a una drástica reducción de la productividad en el sector farmacéutico. Entre ellas cabe citar:

- La inversión en investigación biomédica casi se ha duplicado en la última década y, sin embargo, el número de solicitudes de aprobación de nuevos fármacos se ha reducido a la mitad en el mismo periodo de tiempo, tanto en Europa como en EE. UU.
- La ratio de fracaso y retirada de nuevos compuestos químicos en el proceso de investigación y desarrollo se ha disparado significativamente. En particular, la ratio de fracaso en fase III –la etapa más costosa de todo el proceso de desarrollo– ha aumentado hasta el 50%, cuando históricamente no superaba el 15% (4).
- La incidencia de retirada de productos por motivos de toxicidad, incluyendo la fase IV de poscomercialización, ha aumentado sustancialmente, junto con una reducción marginal de la retirada de productos por problemas de falta de eficacia. Todo lo anterior, después de una década de uso extensivo del nuevo enfoque de investigación basado en las dianas biológicas.
- La decodificación del genoma ha supuesto un espectacular flujo de información segmentada y altamente heterogénea a través de la cual los investigadores no saben navegar de forma

efectiva. Se requiere un nuevo sistema integrado de información en el que interactúen diferentes agentes (públicos y privados) sobre la base del intercambio de datos y experiencias.

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Los avances que se han experimentado en la introducción de fármacos biológicos ofrecen unos beneficios marginales en salud que podrían no corresponderse con el coste de los mismos. Esto conduce a una mayor sensibilidad al precio por parte de los financiadores sanitarios.

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La reducción paulatina en el mercado de productos que ofrecen ingresos sustanciales (por encima de un millardo de dólares anuales) ha supuesto la revisión del modelo de blockbusters , aunque no existe una alternativa clara y de consenso.

Como se indicaba en la sección anterior, la aplicación de las opciones tecnológicas derivadas de la Farmacogenómica se ha centrado principalmente en mejorar el proceso de investigación y desarrollo de nuevos fármacos, con el objetivo de inyectar eficiencia en el sistema. No obstante, existe el ámbito de aplicación en la fase de comercialización del producto, donde incrementos de eficacia garantizados por test diagnósticos se asociarían a grupos más reducidos de pacientes y, por lo tanto, a mercados más restringidos.

En este sentido, la transición que podría experimentar el sector farmacéutico significaría el paso de un modelo basado en blockbusters a un modelo basado en minibusters

.

Estratificación de pacientes versus estratificación de enfermedades

Los avances científicos que permiten comprender mejor los mecanismos que originan las enfermedades, así como identificar mejor las respuestas farmacológicas, crean oportunidades para una correspondencia cada vez más aproximada entre pacientes y terapias. En el punto extremo, cuando esa aproximación es muy acentuada, se habla de medicina individualizada o personalizada. La vacuna anticancerígena Oncophage , actualmente en desarrollo, es un ejemplo de medicina individualizada.

Para producirla se extraen las células cancerígenas del paciente y, tras un proceso específico de elaboración, la vacuna se administra al paciente después de su recuperación de la cirugía. La vacuna, únicamente idónea para ese paciente, estimula una serie de respuestas inmunológicas que atacan a aquellas células cancerígenas que permanecen en el cuerpo.

En otro orden de cosas, se hace referencia a medicina segmentada o estratificada en el caso de aquellas terapias que disponen de un marcador biológico capaz de predecir la respuesta farmacológica de un paciente (5). En este sentido, un paciente puede ser incluido en una cohorte que ha exhibido históricamente una respuesta terapéuti-

ca diferencial, utilizando un biomarcador que se correlaciona con esa respuesta. La clase de biomarcadores que establecen una relación directa entre subpoblaciones de pacientes y tratamientos se denomina biomarcadores clínicos. La introducción de la Farmacogenómica puede implicar un cambio de modelo hacia fármacos dirigidos a subpoblaciones de pacientes (asociados a niveles relativamente altos de eficacia), cuyo impacto en los ingresos financieros de las compañías farmacéuticas estaría por determinar. A este respecto, la estratificación tendría dos vertientes: estratificación de pacientes y estratificación de enfermedades (Tabla III)

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En la primera tipología, el aspecto determinante es la respuesta de los pacientes en términos de seguridad y/o eficacia. La posibilidad de utilizar test diagnósticos que determinen la velocidad en el metabolismo de los fármacos (por ejemplo, identificando el tipo de enzima polimórfica del paciente CYP) permitiría elegir la dosis apropiada para obtener el beneficio terapéutico esperado. Este tipo de estratificación podría incrementar sustancialmente el tamaño del mercado y los ingresos de las compañías farmacéuticas a través de presentaciones de fármacos con dosificaciones alternativas y el aumento de la demanda en pacientes que previamente no obtenían efectos terapéuticos a causa de una dosificación inadecuada. A su vez, estos fármacos no requieren la misma inversión en investigación y desarrollo que la que necesitan los nuevos fármacos.
La segunda tipología de estratificación, la forma más común cuando se hace referencia a la Farmacogenómica, implica la subdivisión en enfermedades. En este caso, a los pacientes con síntomas similares se les prescriben terapias diferentes en función de su perfil molecular. Por ejemplo, pacientes afectadas de cáncer de mama pueden dividirse en dos grupos según el resultado del test diagnóstico: HER2 positivo (con sobreexpresión de la proteí na) y HER2 negativo (sin sobreexpresión de la proteína). Las pacientes del primer grupo serían candidatas a recibir quimioterapia basada en el anticuerpo monoclonal Herceptin (trastuzumab); por el contrario, las del segundo grupo recibirían quimioterapia estándar, ya que su perfil genómico supone la inefectividad del tratamiento con Herceptin (trastuzumab). Ello sugiere una transición de productos farmacéuticos dirigidos a poblaciones de pacientes (blockbusters) a productos dirigidos específicamente a subgrupos de pacientes en función del perfil genómico (minibusters)
. Esta transición podría hacerse efectiva por dos motivos. En primer lugar, por las crecientes dificultades que existen en el proceso de investigación y desarrollo del diseño de compuestos químicos ideales para poblaciones de pacientes. En segundo lugar, por la creencia (fundada o infundada) de que mayores garantías de eficacia terapéutica (apoyadas en instrumentos farmacogenómicos) tienen un impacto positivo en los ingresos de la industria farmacéutica en razón de una mayor disposición a pagar y una mayor predisposición al cumplimiento terapéutico. Sin embargo, una serie de factores, que se detallan a continuación en el documento, intervendrían en la viabilidad del nuevo modelo, y algunos sugieren que las compañías farmacéuticas deberían desarrollar fármacos para cada segmento de enfermedad si desean obtener similares niveles de rentabilidad que la obtenida en el modelo de blockbusters
(6).

Criterios para determinar la viabilidad de la segmentación de enfermedades

La medicina estratificada sugiere un nuevo modelo económico para la industria biofarmacéutica. No obstante, para que el nuevo modelo sea viable deben satisfacerse tres condiciones o criterios básicos: disponibilidad de instrumentos efectivos y de condiciones para identificar a subpoblaciones de pacientes; atractivo económico; y, finalmente, existencia de factores para la sostenibilidad del nuevo modelo.
Factibilidad de identificar a subgrupos de pacientes Uno de los factores clave para discriminar a pacientes radica en un efectivo biomarcador clínico. Si la ausencia de un biomarcador validado fuera el único factor responsable, a medida que progresara el avance científico la mayoría de las áreas terapéuticas evolucionarían hacia la medicina estratificada. Sin embargo, éste no es el caso; son necesarios una serie de factores para que surjan subclases de pacientes con relevancia clínica (5):

- Variabilidad asociada a la enfermedad y que refleje una etiología multifactorial.
- Multiplicidad de dianas biológicas.

Tabla III.
Tipos de estratificación derivados de la Farmacogenómica y sus consecuencias
Estratificación de pacientes Estratificación de enfermedades
Diferentes dosis en función del perfil genómico del paciente Diferentes terapias en función del perfil genómico del paciente
Puede incrementar el tamaño del mercado Puede reducir el tamaño del mercado para un fármaco individual
Se mantiene compatible con el modelo de blockbusters
El objetivo es crear un minibuster para cada área terapéutica .
Fuente: Shah (6).

- Características diferenciales en ADME (absorción, distribución, metabolismo y excreción), en toxicidad y en tolerabilidad a los regímenes terapéuticos.
- Adaptabilidad de la enfermedad que conduce a resistencias al tratamiento.
- Múltiples opciones terapéuticas asociadas con respuestas a la enfermedad heterogéneas.
- Disponibilidad de biomarcadores aceptables en términos médicos y logísticos.

Atractivo económico Para que un fármaco muestre viabilidad económica, debe aportar al menos unos ingresos financieros de 500 millones de dólares anuales con objeto de recuperar los costes hundidos de investigación y desarrollo (Grabowski y Vernon, 2000). No obstante, la incorporación de las opciones farmacogenómicas al modelo puede suponer una revisión de los actuales criterios de viabilidad económica.

En efecto, los costes de I+D probablemente serán inferiores, porque la estratificación y el diferencial de eficacia pueden reducir sustancialmente el número, el tamaño y los tiempos requeridos para los ensayos clínicos: de 10-12 años a 3-5 años con el nuevo modelo (7). Por ejemplo, Glivec (imatinib) fue aprobado por la FDA en sólo tres meses para abordar la leucemia mieloide crónica, con un total de tiempo transcurrido de cuatro años desde la determinación de la primera dosis en los humanos hasta la aprobación por parte del regulador. Adicionalmente, la aplicación farmacogenómica en fases de desarrollo puede reducir significativamente la ratio de fracaso de los nuevos compuestos. El coste asociado al fracaso y a la retirada de compuestos clínicos, junto con el coste de oportunidad de capital, representa el 80% del coste total del desarrollo de nuevos fármacos (8).

En la fase de comercialización, la provisión simultánea de test diagnóstico y terapia farmacológica puede generar significativos ingresos financieros por las siguientes razones: eficacia clínica relativamente superior; periodo efectivo de patente más extenso, derivado de los tiempos más reducidos de los ensayos clínicos; ingresos adicionales originados por la comercialización del biomarcador; mayor sensibilidad de reguladores y pacientes a aceptar precios más altos por una mayor efectividad terapéutica.

Los productos biológicos trastuzumab (Herceptin Roche) e imatinib (Glivec , Novartis) ejemplifican la potencialidad del modelo de estratificación de enfermedades. Sin embargo, el fármaco para la antisepsis drotrecogina alfa (Xigris , Eli Lilly) es un ejemplo de fracaso de

acuerdo con sus expectativas iniciales de ingresos, debido a un segmento de pacientes demasiado reducido (pacientes con sepsis severa).

Existencia de factores para la sostenibilidad del nuevo modelo

A fin de que la medicina estratificada sea viable económicamente, se requiere un significativo grado de adopción de los productos generados dentro de cada subgrupode pacientes. Para ello se necesita desplazar sustancialmente las terapias vigentes y levantar el "listón" de la efectividad terapéutica a un nivel que sirva de barrera de entrada para los competidores.

Los factores que contribuyen a la ventaja competitiva son:

- Mejoras en el cumplimiento terapéutico debido a la percepción de los pacientes de una mejor respuesta a la terapia farmacológica. Es particularmente relevante en el caso de las enfermedades crónicas.
- Discriminación positiva de medidas reguladoras para incentivar los avances terapéuticos en poblaciones de pacientes de reducido tamaño. Concretamente, la aplicación del estatus de fármacos "huérfanos" a los productos derivados de la Farmacogenómica.
- Refuerzo de los derechos de propiedad intelectual aplicados a dianas y terapias biológicas.
- Percepción por parte de la clase médica de la bondad y de la utilidad clínica de los biomarcadores como instrumentos de ayuda a la prescripción.

Sin embargo, esta percepción requiere una inversión previa en formación para adaptar al colectivo médico al nuevo modelo.

Cabe señalar que, hasta el momento, la medicina estratificada ha generado productos altamente diferenciados. Si ese nivel de diferenciación no es posible mantenerlo en el medio y largo plazo, los beneficios derivados de la rápida adopción y de la disponibilidad a pagar precios altos podrían ser muy inferiores a los esperados.

Obstáculos para la transición de modelo

La transición al nuevo modelo no está exenta de dificultades. Una vez que se ha conseguido identificar la interrelación entre un gen y una enfermedad, el desarrollo de un test diagnóstico lleva su tiempo. Aun cuando este instrumento está comercialmente disponible, se observa un alto grado de infrautilización. Un ejemplo ilustrativo es la aplicación de test genéticos para determinar la dosis óptima del anticoagulante oral acenocumarol (Sintrom).

Se trata de una terapia cuyos efectos adversos en EE. UU. provocan un coste anual de 1,1 millardos de dólares, y cuya aplicación del test diagnóstico a la población de pacientes de riesgo se estima en un coste anual de sólo 160 millones de dólares. Hasta la fecha, en EE. UU. sólo a un 5% de los pacientes a los que se les administra el anticoagulante oral se les aplica también el test diagnóstico (9).

Las principales barreras que debe superar el modelo para alcanzar su consolidación son: resistencias de la industria farmacéutica a abandonar el modelo de blockbusters; una estructura reguladora que no está adaptada a las necesidades del nuevo modelo; sistemas de financiación que priorizan procedimientos curativos por encima de los de diagnóstico y prevención; cultura y formación médica que no están adaptadas a la revolución genómica; y, finalmente, sistemas de información segmentados e incapaces de resolver las necesidades

del nuevo enfoque de investigación y desarrollo de fármacos.

Resistencia de la industria farmacéutica a abandonar el modelo de blockbusters.

Desde mediados del siglo xx hasta finales de los 90, el modelo centrado en el descubrimiento y comercialización de productos capaces de generar altos ingresos ha funcionado de manera satisfactoria. En efecto, la industria farmacéutica es uno de los sectores más rentables de la economía mundial. A pesar de que todos los indicadores muestran desde el inicio de la década actual una sustancial caída de la productividad en el sector, la reacción de la mayoría de las grandes compañías ha sido reacomodarse en el actual modelo en lugar de sustituirlo. Las oleadas de fusiones, adquisiciones, obtención de licencias de compuestos químicos en fases tempranas del desarrollo, así como los acuerdos de colaboración entre grandes compañías y pequeñas biotecnológicas, reflejan un cambio de orientación, pero no de modelo. Asimismo, una parte significativa de la industria se muestra reacia a la provisión conjunta de test diagnósticos y terapias farmacológicas, debido a que el nuevo componente añade complejidades reguladoras, de marketing y de adaptación por parte de los proveedores asistenciales. Asimismo, un sector acostumbrado a tener el viento a favor como es éste se muestra reacio a exponerse a los riesgos asociados con un cambio de modelo. Si a ello se añade que la aplicación de la Farmacogenómica no se apoya en una evidencia empírica consolidada, esas resistencias tienen cierta base.

Ausencia de adaptación de la estructura reguladora a las necesidades del nuevo modelo

Los pasos que se han dado en este sentido por parte de la FDA en EE. UU. apuntan a incentivar a la industria farmacéutica a utilizar opciones tecnológicas de la Farmacogenómica (en particular, biomarcadores) para inyectar eficacia en los ensayos clínicos y acelerar el procedimiento de aprobación de nuevos fármacos. Las iniciativas Critical Path Initiative Y Voluntary Genomic Data Submission son reflejos de este esfuerzo. Por su parte, la European Medicines Agency (EMEA) ha centrado su actividad en la publicación de un documento de estandarización de terminología genómica como paso previo para las consultas con la industria farmacéutica y con las agencias de evaluación nacionales.

No obstante, el enfoque de ambas entidades para abordar aspectos reguladores que se refieren a test genéticos, ensayos clínicos, licencias y nuevas indicaciones no está claro. En particular, las complejidades reguladoras asociadas a un enfoque de evaluación y aprobación conjunta de test diagnóstico y terapia farmacológica permanecen en una situación de indefinición alarmante, a pesar de que existen casos consolidados en el mercado de este tipo de combinación (por ejemplo, Herceptin y Glivec) y un número creciente en desarrollo.

Este vacío regulador se explica en parte por la difícil transición que supone pasar de aprobar un fármaco para una indicación específica a aprobar un producto (combinado con un test diagnóstico) para una indicación y sujeto a la recomendación para su uso en pacientes con un perfil genómico específico. El que la asociación entre perfil genómico y respuesta farmacológica sea probabilística acentúa la complejidad.

Por otra parte, la posibilidad de que los fármacos originados por la medicina estratificada puedan beneficiarse del estatus de fármacos "huérfanos" requiere un posicionamiento claro del agente regulador. En el caso de la FDA, las decisiones poco fundamentadas de otorgar ese estatus a imatinib (Glivec Novartis) y de rechazarlo en trastuzumab (Herceptin , Roche/Genentech) provocan incertidumbre en la industria farmacéutica. Finalmente, los condicionantes éticos ligados a la recogida, almacenamiento y uso de datos genéticos son legítimos, pero actúan como obstáculos para la innovación en este campo.

Son precisas definiciones, respuestas y actuaciones efectivas de los agentes reguladores para solventar ese dilema.

Procedimientos de financiación que no incentivan el diagnóstico y la prevención La problemática de sistemas de financiación hospitalarios que orbitan sobre la actividad

asistencial meramente de carácter curativo es común tanto en los sistemas públicos de salud como en los privados. Asimismo, los presupuestos sanitarios (en los sistemas públicos) y los contratos de financiación por actividad (en el sector privado) se basan invariablemente en compartimentos estancos y en el cortoplacismo. En este sentido, todo programa de prevención o de diagnóstico precoz que se lleve a cabo en la Atención Primaria tendrá en el medio o largo plazo un impacto económico negativo en el presupuesto de la atención especializada, dado que reducirá la actividad y, consecuentemente, la financiación en esta última.

En el caso norteamericano, la financiación de los programas estatales Medicare y Medicaid se centra en el "pago por procedimiento" y se gestiona a través de un sistema de codificación de procedimientos que supervisa un comité de asesores de diagnósticos médicos sin representación de la industria. Cada vez que se introduce una nueva técnica de diagnóstico (por ejemplo, un test diagnóstico para identificar el perfil genómico), comienza un proceso de solicitud altamente burocratizado que puede des-

incentivar la innovación (9). Como consecuencia, el sistema de reembolso premia a los médicos por los tratamientos y por las intervenciones quirúrgicas que realiza, y a su vez los penaliza desde el punto de vista financiero por el tiempo y el esfuerzo necesarios para obtener un diagnóstico preciso.

A este respecto, la infrautilización tanto en Europa como en EE. UU. de productos derivados de la Farmacogenómica, que pueden suponer un ahorro sustancial de recursos para el sistema en su conjunto (por ejemplo, el anticoagulante warfarina en EE. UU. y la reducida tasa de aplicación del test IHQ y del test FISH para la detección de HER2 positivo en el Reino Unido), puede deberse en parte a la orientación y a los objetivos de los sistemas de financiación vigentes.

Ausencia de una cultura y de una formación médica adaptadas a la revolución genómica

El reto de actualizar el conocimiento sobre Genómica y Proteómica del colectivo médico es enorme.

En EE. UU. la mayoría de las Facultades de Medicina no han incorporado plenamente estas nuevas áreas del saber a sus programas académicos. En lo que se refiere a la cultura actual de los médicos, tanto en Europa como en EE. UU. las organizaciones médicas históricamente han mostrado resistencia a la hora de recomendar nuevas guías y estándares de actuación que reducen la libertad en la práctica médica.

Los test diagnósticos en Farmacogenómica no siempre ofrecen una repuesta categórica de tipo "Sí" o "No" cuando se prescribe un tratamiento; ello añade una dificultad mayor a las posibilidades de que los médicos acepten los instrumentos farmacogenómicos en su práctica habitual. En este sentido, las principales barreras para adoptar la Farmacogenómica serían: la escasez de evidencia científica sobre los beneficios y sobre la utilidad clínica de los biomarcadores clínicos; la preocupación de que suponga una carga de trabajo adicional para el médico prescriptor; los pros y los contras de sustituir una práctica clínica basada en el trial and error por otra afectada también por un grado significativo de incertidumbre.

Sistemas de información segmentados e ineficaces para el reto de la Farmacogenómica

Una de las consecuencias de la decodificación del genoma humano ha sido el increíble volumen de información heterogénea que ha generado y que, según los expertos, ha transformado la Biología en una ciencia de la información (4). Los investigadores se enfrentan al reto de transformar información en conocimiento sobre qué genes afectan a la respuesta farmacológica, sobre cómo afecta eso al resultado de la enfermedad y sobre cómo identificar a

los pacientes en los que se obtendrían los mejores resultados. En gran medida, lo anterior tiene su respuesta en los sistemas de información, pero el problema reside en que parte de esa información necesaria no existe o está segmentada y en que parte de la existente es difícilmente comprensible.
El reto está en crear sistemas integrados de información en los que participen compañías farmacéuticas, biobancos, agencias reguladoras, centros especializados en tecnologías de la información, hospitales y universidades. El objetivo es pasar de un sistema de derechos de propiedad sobre la información a un sistema cooperativo de intercambio de información. Una de las primeras iniciativas ha sido la creación en el 2005 del Predictive Safety Testing Consortium , que tiene como objetivo compartir datos y experiencias sobre métodos de laboratorio para predecir problemas de toxicidad en nuevos fármacos antes de que entren en la fase clínica. Seis compañías farmacéuticas (Bristol Myers-Squibb, GlaxoSmithKline, Johnson & Johnson, Merck, Pfizer y Schering-Plough), la Universidad de Arizona y la FDA forman el consorcio.

EL IMPACTO ECONÓMICO DE LA FARMACOGENÓMICA EN LOS SISTEMAS SANITARIOS

Se ha indicado que la aplicación de las opciones tecnológicas de la Farmacogenómica hasta la fecha se ha centrado principalmente en la mejora de la eficacia de los procesos de descubrimiento, investigación y desarrollo de nuevos fármacos. No obstante, la incipiente introducción de test diagnósticos farmacogenómicos en la práctica clínica vislumbra unos potenciales beneficios clínicos y económicos en el corto y largo plazo de gran relevancia para los sistemas sanitarios. Asimismo, conviene analizar en detalle cuáles son los factores que garantizarían una relación coste-efectividad adecuada de la combinación de test diagnóstico y terapia , y, en consecuencia, su consolidación en la práctica clínica habitual. La extensión del uso de algunos productos farmacogenómicos permite mostrar ejemplos prácticos de la aplicación de la evaluación económica en el campo de la Farmacogenómica.

Beneficios clínicos y económicos asociados a la Farmacogenómica

La incorporación de la Farmacogenómica en el sector sanitario ofrece oportunidades potenciales en el corto plazo de mejora de la salud de los pacientes y de seguridad a través de la reducción de los efectos adversos y de las mejoras en efectividad de las terapias farmacológicas.
En lo que se refiere al largo plazo, los beneficios potenciales apuntan a reducciones en la carga de la enfermedad, a mejoras en la eficiencia de los sistemas sanitarios y a la reducción de las disparidades en el acceso a cuidados de salud.

Beneficios esperados a corto plazo La práctica clínica tradicional de trial and error produce en EE. UU. tres millones anuales de incorrectas e inefectivas prescripciones de fármacos. En el 2001 los efectos adversos asociados a los fármacos afectaron a 2,2 millones de ciudadanos en ese país; de ellos, 106.000 fueron víctimas mortales. Representan la quinta causa de mortalidad en EE. UU. La carga económica asociada a los efectos adversos es muy significativa; se estima un coste anual de 177 millardos de dólares (10). Debido a que las

variaciones genéticas relacionadas con la actividad enzimática que se genera en el metabolismo de los fármacos están altamente correlacionadas con los efectos adversos, la aplicación de la Farmacogenómica (por ejemplo, test diagnósticos) es potencialmente beneficiosa. Aun consiguiendo resultados modestos en la reducción de la ratio de efectos adversos, puede producir mejoras sustanciales en cuanto a resultados clínicos y costes. El caso de la enzima conocida como CYP2D6 es un ejemplo ilustrativo de la aplicación actual y futura de la Farmacogenómica,
puesto que esta enzima metaboliza un número significativo de fármacos con un alto volumen de prescripción en Europa y en EE. UU.
En relación con la efectividad, de las 14 clases principales de fármacos actuales siete han demostrado una respuesta efectiva en menos del 50% de los pacientes. Los fármacos más comunes para el abordaje de la diabetes, la depresión y el asma son efectivos aproximadamente en el 60% de los pacientes en tratamiento; y, respecto a las terapias anticancerígenas, su efectividad se reduce al 25% de los pacientes (11). Los biomarcadores clínicos para la estratificación de pacientes y enfermedades permitirían redirigir tratamientos de alto coste (por ejemplo, productos biológicos aplicados a enfermedades crónicas o en vías de cronificación) exclusivamente a subgrupos de pacientes cuya respuesta fuese positiva, evitando el derroche de recursos.
Beneficios esperados a largo plazo La importancia relativa de las enfermedades crónicas está creciendo en los países desarrollados; en EE. UU. Se estima que más de 134 millones de ciudadanos se verán afectados por alguna enfermedad crónica en el 2020 (2).
Los tratamientos actuales para el abordaje de estas enfermedades consiguen, en el mejor de los casos, desacelerar la progresión de las mismas y reducir los síntomas.
Por su parte, los objetivos de los productos derivados de la Biotecnología son menos modestos y apuntan a una reducción de la carga y prevalencia de la enfermedad, y, en particular, a fomentar el cumplimiento de la administración farmacológica para alcanzar mayores niveles de efectividad.
La selección de terapias en función de la probabilidad de respuesta positiva mediante instrumentos farmacogenómicos puede reducir los problemas de adherencia.
Es un tema relevante, ya que la mitad de los pacientes afectados por condiciones crónicas abandonan voluntariamente el tratamiento después de un año (2).
No obstante, el aspecto de mayor potencialidad de la Farmacogenómica se centra en la prevención y mejora del diagnóstico. La detección de grupos de pacientes de riesgo a través de la identificación del perfil genético es como una carga de profundidad en los cimientos en los que se asientan los actuales sistemas sanitarios. La Farmacogenómica puede ser el motor de la transformación de sistemas sanitarios centrados en la actividad curativa (enfoque reactivo) a sistemas de cuidados de salud centrados en la prevención (enfoque predictivo).
Los costes a corto plazo pueden desincentivar esa transformación: costes de adaptación de la práctica médica a los test diagnósticos; costes de formación para actualizar a los médicos en el conocimiento sobre Genómica y Proteómica; costes de introducción y financiación de los test diagnósticos; precios más altos de las terapias de mayor efectividad. Y, sin embargo, los efectos positivos en el largo plazo en términos clínicos y económicos de la prevención por vía farmacogenómica no admiten dudas, siempre y cuando se abandone la actitud cortoplacista.

El impacto económico de la medicina estratificada: una simulación

Las potencialidades de la Farmacogenómica en cuanto a incrementar la eficiencia en el proceso de investigación y desarrollo de nuevos fármacos pueden tener un impacto

económico para la industria farmacéutica que no es fácil de evaluar. Una primera aproximación es la simulación propuesta por Trusheimet al . (5) que se representa en la Tabla IV
. En la columna de "Actualidad" se muestran unas razonables ventas anuales, estimadas en 500 millones de dólares, para un fármaco de éxito que ha supuesto una inversión en I+D de 1.000 millones de dólares (8). Con un periodo restante de patente de diez años y con un margen bruto actual del 80%, se generan 4.000 millones de dólares en diez años, que dan lugar a una ratio de rentabilidad de 4 (beneficio bruto/costes de desarrollo). A la luz del incremento continuado de la ratio de fracaso en las fases clínica y preclínica, el diagnóstico sobre la evolución de los costes del desarrollo de nuevos fármacos sugiere un incremento del 100% de esos costes y un incremento del periodo efectivo de desarrollo del 40%; de 1.000 a 2.000 millones de dólares de coste medio de un fármaco en desarrollo; y de 10 a 14 años de tiempo de I+D (4). Teniendo en cuenta esta tendencia a corto-medio plazo, es factible prever un declive en los márgenes brutos de la industria farmacéutica hasta el 50%; margen que en cualquier caso se mantiene por encima del 33% que exhiben las compañías de genéricos en EE. UU. (12). En estas circunstancias, aun cuadriplicándose el volumen de ventas anuales, la ratio de rentabilidad caería del 4 al 3 y provocaría un declive industrial acentuado.

Por su parte, se estima que la introducción y la extensión de los instrumentos farmacogenómicos para mejorar la eficacia de los ensayos clínicos podrían reducir a la mitad el periodo medio de I+D a cinco años (7) y, en un escenario conservador, estabilizar la tendencia de crecimiento (diez años). El uso de biomarcardores, con la consecuente reducción de las ratios de fracaso y de la duración de los ensayos clínicos, podría disminuir hasta un 75% los costes de I+D (50% en un escenario conservador).
En relación con las ventas, y a la vista de los precios de reembolso que se están aplicando a los productos biológicos, la introducción de los minibusters podría generar unas ventas anuales de 200 millones de dólares. La previsión de volumen de ventas podría ser superior si se añade la comercialización de los test diagnósticos. Todo lo anterior podría hasta duplicar la ratio de rentabilidad de 4 a 9,6 o, en un escenario más conservador, evitar su declive actual y estabilizarlo en 4,3. Sin embargo, la creciente sensibilidad al precio de las autoridades sanitarias podría conducir a un futuro todavía más conservador que el simulado por Trusheim et al . (5).

Factores que influyen en el análisis coste-efectividad de los productos farmacogenómicos

La viabilidad económica de la combinación test diagnóstico y terapia farmacológica dependerá de las circunstancias específicas de su uso. La evidencia científica concluye que el screening médico se justifica únicamente cuando se centra en grupos de alto riesgo (13). En términos generales, los test diagnósticos son económicamente viables antes del tratamiento si los ahorros generados al evitar tratamientos ineficaces y efectos adversos son superiores a los costes de su implantación.
Las incursiones en la evaluación económica aplicada a la introducción de test diagnósticos farmocogenómicos como mecanismo de selección de terapias comparan dos estrategias clínicas alternativas (14-17):
1.

Aplicar el test diagnóstico a toda la población de pacientes para determinar si se prescribe la terapia de referencia (por ejemplo, un fármaco biológico) o un tratamiento de primera línea. Si el resultado del test es negativo, se prescribe una terapia convencional o de segunda línea.
2.
No aplicar el test diagnóstico y prescribir la terapia de referencia.
En las experiencias de análisis coste-efectividad, se observa la inclusión de una o más estrategias clínicas que incluyen la aplicación de test diagnósticos con características diferentes (por ejemplo, sensibilidad y especificidad). Asimismo, se añaden estrategias que reflejan la aplicación simultánea de dos test diagnósticos para abordar la discordancia de resultados.
En la Tabla V se muestra la relación de variables que pueden influir, en mayor o en menor medida, en el resultado de un análisis coste-efectividad donde se comparan las dos alternativas previamente especificadas. Igualmente, para cada variable se indica cuál sería el Impacto individual (cæteris paribus) en la ratio coste-efectividad incremental (RCEI).
En lo que respecta a la prevalencia (porcentaje de pacientes con una mutación genética) y a la Penetrancia (porcentaje de pacientes con una mutación genética que se verán afectados por la enfermedad asociada) del perfil genético, valores relativamente altos de esos indicadores tendrán un efecto individual positivo en la RCEI y, en consecuencia, en la elección de la alternativa "test diagnóstico en la población de pacientes". En este sentido, una prevalencia alta de no respuesta supondría que Tabla IV.
Simulación del impacto de la Farmacogenómica en la I+D de nuevos fármacos
Parámetro Actualidad Tendencia Futuro optimista Futuro realista
Periodo de patente* 20 20 20 20
Tiempo de desarrollo* 10 14 5 10
Costes de desarrollo** 1.000 2.000 250 500
Periodo de comercialización* 10 6 15 10
Media de ventas anual** 500 2.000 200 330
Margen bruto 80% 50% 80% 65%
Beneficio bruto** 4.000 6.000 2.400 2.150
Ratio de rentabilidad*** 4 3 9,6 4,3
* En años.
** En millones de dólares.
*** Beneficio bruto/costes de desarrollo.
Fuente: Trusheim
et al.
(5).

con la aplicación del test se podría evitar un coste inefectivo superior de un mayor número relativo de pacientes (que no responden) y, por tanto, afectaría positivamente la RCEI. En el estudio de Stallings et al . (17) cuyo objetivo es elegir tratamientos en enfermos de asma, la variable prevalencia de no respuesta es un factor determinante en el resultado, junto con el precio del test y el coste de errar el tratamiento en términos de carga de enfermedad adicional y efectos adversos. En relación con la penetrancia, su efecto va en una dirección similar.
Es decir, cuanto mayor sea el porcentaje de pacientes que van a sufrir la enfermedad, mayor será el rendimiento de la inversión, en términos de efectos en salud, de generalizar el test diagnóstico.
En cuanto a las características del test diagnóstico (sensibilidad : probabilidad de que el test sea positivo si la persona tiene una mutación; y especificidad : probabilidad de que el test sea negativo si la persona no la tiene), cuanto mayores sean estas probabilidades, más probable

será que la estrategia "test para población de pacientes" exhiba una mejor relación coste-efectividad.
En el estudio de Elkin et al . (14), que compara hasta siete estrategias de aplicación de test diagnósticos para discriminar el subgrupo de pacientes con cáncer de mama HER2 positivo, las variaciones en las características del test de referencia IHQ en el análisis de sensibilidad provocan las modificaciones más drásticas en el ranking coste-efectividad incremental de estrategias. En particular, se observa que, a menos que la sensibilidad del test IHQ supere el 96%, la estrategia alternativa de emplear el test más caro FISH (un 20%-30% más caro que IHQ) tendrá una mejor relación coste-efectividad.
En referencia al coste del test diagnóstico , los cuatro estudios analizados sugieren que es, junto con la prevalencia, la variable que mayor impacto tiene en la RCEI; con la excepción del análisis de Elkin et al. (14), que requiere un diferencial de precios muy elevado entre test alternativos para provocar cambios en el ranking de estrategias.
Sorprendentemente, la variable coste del tratamiento de referencia Rx1 no parece tener un efecto significativo en los resultados. En el estudio de Morelle et al . (16) una disminución del precio de Herceptin (trastuzumab) del 50% provoca las reducciones más notables en las magnitudes de la RCEI para cada estrategia, pero mantiene invariable el ranking coste-efectividad.

Aplicación práctica del análisis coste-efectividad en Farmacogenómica

Los test diagnósticos empleados para determinar el estatus HER2 en el abordaje del cáncer de mama constituyen uno de los pocos ejemplos, hasta la fecha, de aplica

Tabla V.
Factores que influyen en el análisis coste-efectividad referido a la introducción de un test diagnóstico prepresCripción y su impacto esperado en la RCEI

Aplicación del test diagnóstico vs . no aplicación	Impacto individual (+) (reducción de la RCEI)	Impacto individual (–) (incremento de la RCEI)
Prevalencia del perfil genético de no respuesta al tratamiento Rx1	Alta	Baja
Asociación entre mutación genética y enfermedad (penetrancia)	Alta	Baja
Ratio de respuesta a la terapia de referencia Rx1	Alta	Baja
Ratio de respuesta a la terapia alternativa Rx2	Baja	Alta
Sensibilidad del test diagnóstico	Alta	Baja
Especificidad del test diagnóstico	Alta	Baja
Coste del test diagnóstico	Bajo	Alto
Coste del tratamiento de referencia Rx1	Bajo	Alto
Coste del tratamiento alternativo Rx2	Alto	Bajo
Diferencial de costes entre Rx1 y Rx2	Bajo	Alto
Tiempo para detectar la enfermedad	Menor	Mayor
Tiempo transcurrido con el tratamiento Rx1 que resulta inefectivo	Menor	Mayor
Efectos adversos con el tratamiento Rx1 inefectivo	Existencia	Inexistencia
Severidad de los efectos adversos	Mayor	Menor
Expectativa de vida y calidad de la misma	Alta	Baja
Costes indirectos	Bajos	Altos

RCEI: ratio coste-efectividad incremental.
Fuente: elaboración propia.

ción práctica de la evaluación económica en el campo de la Farmacogenómica. El relativamente costoso anticuerpo monoclonal Herceptin (trastuzumab) puede ser dirigido a subgrupos de respuesta alta; es, por sí misma, una actuación médica fundamentada en criterios de efectividad.
Aunque existen ejemplos de análisis coste-efectividad para el cáncer de mama precoz con Herceptin (trastuzumab), que implican cambios de gran magnitud en la esperanza de vida y en las curaciones, se ha escogido el estudio de Elkin et al. (14) sobre cáncer metastásico porque incluye la comparación de estrategias de test diagnósticos.
Introducción a los tipos de test diagnósticos La IHQ y la FISH son los métodos más utilizados para el estudio de HER2. Mediante la IHQ se evalúa el grado de expresión de la proteína en la membrana celular y mediante la FISH se evalúa el número de copias del gen en

el núcleo celular para detectar su amplificación. Mientras que muchos laboratorios de patología han incorporado la IHQ, la técnica FISH es menos asequible, requiere un equipamiento más costoso y es difícil de implementar.
En principio, existe consenso sobre la mayor fiabilidad de la técnica FISH, pero en los ensayos clínicos se ha utilizado preferentemente la IHQ y, en algunos de ellos, se han detectado importantes discordancias entre los resultados del primer laboratorio que efectuaba el test y los de un laboratorio de referencia de mayor volumen que lo repetía con posterioridad. En un 20% de los casos el primer resultado se consideró incorrecto (18). Esta variabilidad de resultados se atribuye a factores preanalíticos, como la fijación y el procesado tisular, a factores analíticos, como los reactivos o los procedimientos del laboratorio, y a factores postanalíticos, como los criterios de interpretación de los resultados.
Los algoritmos de decisión terapéutica consensuados y empleados hasta la actualidad, que asumen un alto nivel de concordancia entre IHQ y FISH que la literatura disponible no justifica claramente, recomiendan la IHQ como test primario e indican la FISH sólo en los casos de positividades intermedias no concluyentes (2+). Los casos de positividad alta (3+) se consideran candidatos a trastuzumab y los de positividad baja (1+) o nula se estiman negativos y no se consideran susceptibles de respuesta. En EE. UU. el precio de la IHQ es de 85 dólares (en Francia, de 43 euros); y el de la FISH, de 381 dólares (en Francia, de 283 euros) (16).
Características y suposiciones del modelo
El modelo de Markov desarrollado por Elkin et al. (14) en el escenario norteamericano se compone de cinco estados de salud definidos (cáncer de mama metastásico, enfermedad estabilizada, respuesta al tratamiento, progresión de la enfermedad y muerte). Los pacientes en estado metastásico entran en el modelo con un tratamiento de primera línea. Pueden experimentar respuesta a la terapia, enfermedad estable o progresión de la enfermedad. La mortalidad por cáncer de mama es posible únicamente desde el estado de progresión de la enfermedad.
El objetivo es evaluar la efectividad a largo plazo y el coste de siete estrategias para identificar y tratar a pacientes con cáncer de mama metastásico. Los test diagnósticos y sus combinaciones pretenden identificar a pacientes con amplificación del gen HER2+ a las que el tratamiento con Herceptin ® (trastuzumab) es efectivo (20%-25% de los pacientes).
El modelo de decisión simula resultados en términos de efectos en salud (años de vida ajustados por calidad) y de coste para cada estrategia en una cohorte hipotética de mujeres mayores de 65 años. Se asume que cualquier respuesta objetiva a la terapia ocurrirá dentro de las primeras 18 semanas de tratamiento. Una vez que la paciente entre en el estado de progresión de la enfermedad cesa la aplicación de la terapia de primera línea; finalmente, ninguna paciente recibe más de ocho ciclos de quimioterapia.
Resultados del estudio El análisis coste-efectividad incremental, que se muestra en la Tabla VI , se traduce en un ranking de las siete estrategias ordenadas en función de una efectividad creciente. Tras eliminar las estrategias que muestran un coste superior o igual y una efectividad menor que la estrategia más competitiva (es decir, descartadas por dominancia simple), la ratio incremental (RCEI) de cada estrategia se calcula como el coste adicional de esa estrategia dividido por el beneficio clínico adicional respecto de la siguiente estrategia más efectiva. Si la estrategia resulta ser menos efectiva y exhibe una RCEI superior, se descarta por dominancia extendida. Las estrategias con dominancia extendida se descartan y las RCEI de las restantes estrategias se vuelven a calcular.
Los resultados del estudio son los siguientes:

- La estrategia con un mejor relación coste-efectividad conlleva la aplicación inicial del test IHQ

para todos los pacientes y el empleo posterior del test FISH para confirmar el resultado (2+) (siempre dudoso mediante IHQ) y el resultado (3+) (que no juzgan dudoso las guías clínicas de consenso).
Este resultado es similar al obtenido en el estudio de Dendukuri et al . (15) en el escenario canadiense y al obtenido en el estudio de Morelle et al . (16) en el sistema sanitario francés.
La segunda mejor estrategia es la aplicación exclusiva del test FISH, que es relativamente más caro, pero cuyo grado de exactitud es superior al del IHQ, según el consenso clínico.

- El coste de la estrategia de prescribir trastuzumab y quimioterapia estándar sin ningún tipo de discriminación previa (sin test) representa un coste medio por paciente de 79.181 dólares. Es un 32% superior al coste medio por paciente de la estrategia más coste-efectiva de combinación de test y terapia (53.702 dólares), y un 30% superior a la opción de uso exclusivo del test FISH (54.738 dólares). Esta diferencia a favor del test para la preprescripción sugiere un ahorro significativo en el presupuesto sanitario.
- La RCEI de la estrategia más coste-efectiva (125.100 dólares) se situaría por encima del umbral de decisión utilizado en los estudios de evaluación económica aplicados al ámbito norteamericano. En ese contexto, estrategias cuyas RCEI se sitúen por debajo de 50.000 dólares tienen una alta probabilidad de ser aceptadas; en el intervalo de 50.000-100.000 dólares existe espacio para el debate; y RCEI superiores a 100.000 dólares presentan una alta probabilidad de ser rechazadas por los entes financiadores. Sin embargo, se debe subrayar que el estudio en cuestión se centra en el estado metastásico del cáncer de mama, en el que el objetivo de las terapias anticancerígenas es extender la esperanza de vida. El mismo estudio aplicado al cáncer de mama precoz (o en estados avanzados no metastásicos) puede repercutir de manera notable en la RCEI, debido a que la metástasis se pospone en gran medida en el tiempo y debido a la cura efectiva de la enfermedad.
Estudios de evaluación económica de terapia adyuvante con trastuzumab en Europa exhiben RCEI de 2.396 libras esterlinas, muy por debajo del umbral de decisión empleado por el National Institute for Health and Clinical Excellence (NICE) de 30.000 libras esterlinas (19).
- Desde una perspectiva social, la aplicación de test diagnósticos de preprescripción para identificar potenciales candidatos a una terapia biológica anticancerígena influye considerablemente en términos de coste-efectividad. Independientemente del coste

Tabla VI.
Resultados del análisis coste-efectividad en estrategias de test diagnóstico y terapia Tipo de estrategia combinada Meses de vida Años de vida Coste (dólares) RCEI (test diagnóstico + terapia) ajustables ajustables por calidad por calidad
Sin test
Quimioterapia para todos 15,33 1,28 43.314 -
Test IHQ
Quimioterapia y trastuzumab para (3+)
Quimioterapia para el resto 16,14 1,34 51.231 Dominancia extendida
Test IHQ; FISH confirmación para (3+) y (2+)
Quimioterapia y trastuzumab para FISH (+)
Quimioterapia para el resto 16,32 1,36 53.702 125.100 $
Test IHQ; FISH confirmación para (2+)
Quimioterapia y trastuzumab para (3+) y FISH (+)
Quimioterapia para el resto 16,32 1,36 54.056 Dominancia simple

Test IHQ
Quimioterapia y trastuzumab para (3+) y (2+)
Quimioterapia para el resto 16,32 1,36 57.467 Dominancia simple
FISH
Quimioterapia y trastuzumab para FISH (+)
Quimioterapia para el resto 16,41 1,37 54.738 145.400 $
Sin test
Quimioterapia y trastuzumab para todos 16,41 1,37 79.181 Dominancia simple
RCEI: ratio coste-efectividad incremental; IHQ: inmunohistoquímica; FISH: hibridación in situ
fluorescente.

del test, se debe al alto coste que supone tratar a pacientes falsos positivos y a la oportunidad perdida de ofrecer la terapia a pacientes falsos negativos. No obstante, se requiere mayor evidencia empírica para confirmar estos hallazgos.

LA ADAPTACIÓN DE LOS SISTEMAS DE REGULACIÓN A LA FARMACOGENÓMICA

La introducción en el mercado de productos farmacéuticos derivados de la Farmacogenómica va a afectar considerablemente al entorno regulador y a los diferentes agentes del sector sanitario, en particular al colectivo médico y a los pacientes. Las respuestas a este reto no pueden ser simples o unidimensionales, sino que implicarán múltiples factores y una mayor necesidad de coordinación principalmente entre la industria farmacéutica, los responsables de evaluar fármacos y los responsables de evaluar test genéticos.
La necesidad de adaptar el conocimiento y la práctica médica a los nuevos retos de la Farmacogenómica requiere cambios en la formación médica y una mayor implicación de los agentes reguladores traducida en provisión de guías clínicas asociadas al nuevo tipo de prescripción.
Asimismo, la Farmacogenómica supone un cambio en los fundamentos actuales de los sistemas sanitarios desde un enfoque reactivo a otro predictivo.

Aspectos de regulación relacionados con la investigación y con el desarrollo de fármacos Evaluación y aprobación conjunta de test y fármacos

La nueva frontera está en que los agentes reguladores establezcan guías de consenso para que la industria farmacéutica provea datos farmacogenómicos durante el proceso de desarrollo de fármacos, así como datos asociados a los test genéticos. La FDA ha creado recientemente la Office of Combination Products con la finalidad de abordar este campo (20). En el 2003 se dio un paso similar en el Reino Unido con la fusión de la Medicines Control Agency (responsable de supervisar nuevos fármacos) y la Medical Devices Agency (responsable de supervisar test genéticos), de la que resultó la Medicines and Healthcare Products Regulatory Agency (MHPRA). La variabilidad genética en diferentes poblaciones y su impacto en la respuesta farmacológica requieren la coordinación entre las agencias evaluadoras de los diferentes países. Uno de los primeros pasos en esta línea han sido los contactos entre la MHPRA, la EMEA, la FDA y la Agencia Japonesa de Evaluación de Fármacos. Asimismo, es preciso establecer un nuevo marco regulador que incentive el diseño de ensayos clínicos conjuntos, en los que la investigación de carácter predictivo se aplique simultáneamente al fármaco y al biomarcador.

Legislación sobre fármacos "huérfanos" Como se ha comentado anteriormente, el modelo de segmentación de enfermedades y pacientes que fomenta la Farmacogenómica supone la entrada en escena de los minibusters , fármacos dirigidos a subpoblaciones de pacientes cuya rentabilidad en algunos casos es cuestionable. Una de las opciones en debate es la posibilidad de extender la clasificación de fármacos "huérfanos" a los productos farmacogenómicos. Esta posibilidad representa una oportunidad y al mismo tiempo una amenaza, debido a que los fármacos "huérfanos" ofrecen unas ventajas reguladoras (por ejemplo, aprobación rápida, precios relativamente altos, subsidios financieros, etc.) que resultarían inviables para una oleada de minibusters
. En cualquier caso, una regulación clara y específica es altamente necesaria en vista de las decisiones tomadas por la FDA, sin demasiado fundamento, respecto a la aceptación de Glivec ® (imatinib) como fármaco "huérfano" y al rechazo de Herceptin ® (tras-tuzumab) en esta categoría.

Rescate de fármacos: reaprobación restringida La Farmacogenómica abre la posibilidad de que fármacos previamente retirados en fases de desarrollo o en fase de poscomercialización a causa de efectos adversos puedan ser revisados por el regulador sobre la base de la provisión de datos farmacogenómicos.

La propia FDA ha sido la que ha planteado esta posibilidad, dado que reconoce que podría ser una medida demasiado radical el ostracismo de un producto por sus efectos adversos en un subgrupo reducido de pacientes. El concepto de reaprobación restringida en función de identificar a ese subgrupo con un genotipo específico y, por tanto, permitir la prescripción del fármaco a otras subpoblaciones de pacientes sin ese genotipo tiene como precedente el producto Lotronex ® (GlaxoSmithKline) para el tratamiento del intestino irritable. Sin embargo, este tipo de medidas requieren una regulación suplementaria, entre otras cosas para incentivar a las compañías farmacéuticas a rehacer el procedimiento de aprobación y a aportar datos adicionales, así como una compensación por la reducción del periodo de patente que ya han experimentado esos fármacos que se pretende recuperar.

Prescripción electrónica y programas de seguimiento de pacientes Las nuevas tecnologías serán cruciales para gestionar el gran volumen de datos que va a generar la aplicación de la Farmacogenómica. Aunque el análisis del perfil genómico y de la respuesta farmacológica apunta a una reducción de los efectos adversos en fase de poscomercialización, las expectativas po-

drían ser infundadas en algunos casos. Hay que recordar que el nuevo modelo permitirá ensayos clínicos más cortos y con un volumen de pacientes menor. Esto podría suponer mayor presión en la industria para asegurar que los efectos adversos no aparezcan una vez que el fármaco se comercialice. Se requiere, entonces, un mayor grado de coordinación entre compañías farmacéuticas, reguladores y prescriptores en la supervisión de fármacos sobre la base de la introducción de la prescripción electrónica y de sistemas de tracking de pacientes.

Generalización del análisis coste-efectividad.

En el modelo actual el grado de requerimiento de estudios de evaluación económica para la aprobación y el reembolso de productos farmacéuticos dirigidos a poblaciones de pacientes es desigual entre los países.

No obstante, la aplicación de instrumentos farmacogenómicos y su potencial eficacia sólo para subgrupos de pacientes probablemente llevarán a las autoridades sanitarias a una mayor exigencia en la presentación de este tipo de análisis. En este sentido, es preciso establecer

una estandarización de la metodología para que los resultados tengan una transparencia y una credibilidad aceptables y aceptadas por todos los agentes implicados. Esta estandarización es relativamente más compleja al introducir un nuevo elemento: el test diagnóstico de preprescripción.

Gap entre Biología y Química en el proceso de desarrollo de fármacos

En el nuevo modelo de I+D para el descubrimiento de nuevas entidades moleculares, la identificación de nuevas dianas biológicas surge principalmente del mundo académico y/o de centros de investigación públicos. La industria no dispone de tiempo y recursos para centrarse en la investigación básica. El problema es que los académicos disponen de ventaja competitiva en el conocimiento en Biología, pero generalmente tienen lagunas de conocimiento en Química Aplicada. Una cosa es saber identificar una diana biológica y otra muy diferente validarla y determinar el compuesto capaz de interactuar con ella a través del screen ing y de colecciones de compuestos químicos (mo-lecular libraries)
. El conocimiento sobre Química Médica es crucial en esta etapa; el problema reside en que los mejores químicos están en la industria farmacéutica. Por lo tanto, es fundamental establecer una interrelación entre ambos sectores para cerrar ese gap en la investigación. La FDA ha constituido la Critical Path Initiative con el objetivo de identificar los cuellos de botella en el proceso de I+D y fomentar la investigación cooperativa.

Aspectos de regulación asociados a la práctica clínica

Aspectos sobre equidad y ética que requieren una regulación específica La necesidad de obtener información sobre el perfil genético de los pacientes y/o de los beneficiarios de planes de salud es un asunto de gran relevancia no sólo en la investigación y el desarrollo de fármacos, sino también en su utilización en la práctica clínica. Los datos farmacogenómicos, que resultan cruciales para la innovación, se enfrentan a consideraciones éticas relacionadas con el potencial uso de esa información. Las autoridades sanitarias deben regular la recogida y el uso de datos genéticos, de tal manera que se alcance un equilibrio entre el objetivo de innovar y el de garantizar una utilización adecuada y restrictiva de una información altamente sensible. Por otra parte, en sistemas sanitarios gestionados sobre la base de planes de seguros, la aplicación de test diagnósticos plantea incógnitas respecto al impacto en la equidad de acceso.

Se pueden plantear escenarios donde pacientes con genotipos que determinan una baja probabilidad de manifestar una enfermedad, pero que predicen una pobre respuesta al tratamiento, tendrían un riesgo similar o mayor que otros con una alta probabilidad de caer enfermos, pero que predicen una alta respuesta al tratamiento (6). En tales circunstancias, las posibles discriminaciones en cuanto a coberturas asistenciales por parte de las aseguradoras deberían tener un contrapeso en términos de regulación.

Sistemas de financiación no adaptados al enfoque predictivo y diagnóstico de enfermedades Si los actuales sistemas de financiación no se adaptan a los test diagnósticos con objetivo predictivo, difícilmente se consolidarán estas nuevas opciones tecnológicas en los sistemas sanitarios. La financiación no se circunscribe únicamente a reembolsar adecuadamente el test, incluye también el tiempo que requiere el médico para interpretar los resultados e informar al paciente de las opciones terapéuticas derivadas del test, bajo una carga asistencial notable.

Es una de las causas principales de la actual infrautilización de test que han demostrado exactitud y utilidad clínica. Los casos del test previo a la terapia con warfarina en EE. UU. (sólo se aplica el test a un 5%) (9) y el caso del test para trastuzumab en el Reino Unido (sólo

el 35% de los hospitales lo aplican) (21) son ejemplos que conducen a una reflexión. Asimismo, los sistemas de reembolso se centran en procedimientos curativos (tratamiento e intervenciones quirúrgicas) y dejan en un segundo plano los procedimientos diagnósticos.

Gap entre los test diagnósticos y la práctica clínica
Los requerimientos y las acciones de las autoridades sanitarias para regular el uso combinado de test y terapias están, en la actualidad, en estado embrionario. Por ejemplo, el actual procedimiento de aprobación por parte de la FDA de un test para predecir efectos adversos no incluye ninguna recomendación sobre la dosis de la terapia acompañante (2). Sin esa información relevante, la utilidad clínica del test es nula para el médico prescriptor. Por otro lado, conviene decidir dónde debe aplicarse el test. La Atención Primaria va a la cabeza en volumen de prescripciones y cabe evaluar si es el sector apropiado para la introducción y el uso de esta nueva tecnología. En este orden de cosas, la práctica creciente de subcontratación de test diagnósticos a laboratorios especializados está sujeta a una alarmante falta de control por parte del regulador (3).
Formación médica y farmacogenómica El mencionado gap se acentúa cuando el prescriptor no posee los conocimientos necesarios para realizar e interpretar los resultados de los test y juzga como una carga adicional de trabajo el empleo de esta técnica. La adaptación del médico a los nuevos instrumentos farmacogenómicos es, en gran medida, un asunto de educación; se precisa más formación y un cambio cultural en la práctica médica. En EE. UU. los médicos están obligados a realizar anualmente entre 12 y 50 horas de formación continuada para conservar sus licencias. Sin embargo, muy pocos estados establecen créditos de formación en Genómica y test diagnósticos (9). Asimismo, en las Facultades de Medicina no se ha producido hasta la fecha una actualización del conocimiento genómico en los programas de enseñanza. Finalmente, las organizaciones médicas todavía no han dado el paso de desarrollar guías de práctica clínica que integren nuevas terapias farmacológicas, diagnósticos y estándares de calidad para el screening de perfiles genómicos.
Sin una actuación coordinada de agentes reguladores, financiadores y el colectivo médico, es difícil acelerar la adopción de la medicina estratificada y reformar los actuales sistemas de financiación.

CONCLUSIONES

En este estudio se identifican dos mercados prioritarios de interés comercial. El primero de ellos integra aquellos productos aplicados al desarrollo preclínico y clínico de nuevos fármacos. El segundo mercado se refiere ala producción de test diagnósticos para determinar la prescripción de fármacos (principalmente ya comercializados) y para segmentar enfermedades y pacientes.
Los test diagnósticos se aplican mayoritariamente en el área terapéutica del metabolismo (test dirigidos a enzimas que actúan en el metabolismo de los fármacos).
La segunda área terapéutica con mayor aplicación de los test diagnósticos se refiere a los fármacos anticancerígenos. Respecto a los criterios que determinan la viabilidad del modelo de medicina estratificada, son los siguientes: disponibilidad de instrumentos efectivos para identificar subpoblaciones de pacientes, atractivo económico y factores que apoyen la sostenibilidad del nuevo modelo.
Entre estos últimos cabe destacar: en primer lugar, mejoras en el cumplimiento terapéutico debido a la percepción de los pacientes de una mejor respuesta a la terapia farmacológica; en

segundo lugar, la discriminación positiva de medidas reguladoras para incentivar los avances terapéuticos en poblaciones de pacientes de reducido tamaño, concretamente la aplicación del estatus de fármacos "huérfanos"; en tercer lugar, el refuerzo de los derechos de propiedad intelectual aplicados a dianas y terapias biológicas; y, finalmente, la percepción por parte de la clase médica de la bondad y de la utilidad clínica de los biomarcadores como instrumentos de ayuda a la prescripción.

En lo que se refiere al impacto clínico y económico de la medicina estratificada, se observa que la incorporación de la Farmacogenómica en el sector sanitario ofrece oportunidades potenciales en el corto plazo de mejora de la salud de los pacientes y de seguridad a través de la reducción de los efectos adversos y de las mejoras en efectividad de las terapias farmacológicas. Por lo que respecta al largo plazo, los beneficios potenciales apuntan a reducciones en la carga de la enfermedad, a mejoras en la eficiencia de los sistemas sanitarios y a la reducción de las disparidades en el acceso a cuidados de salud.

Las experiencias de evaluación económica muestran que desde una perspectiva social la aplicación de test diagnósticos de preprescripción para identificar potenciales candidatos a recibir una terapia biológica anticancerígena tiene una influencia considerable en términos de coste-efectividad. Independientemente del precio del test, se debe al alto coste que implica tratar pacientes falsos positivos y a la oportunidad perdida de no ofrecer la terapia a pacientes falsos negativos.

Finalmente, la introducción en el mercado de productos farmacéuticos derivados de la farmacogenómica va a afectar considerablemente al entorno regulador y a los diferentes agentes del sector sanitario, en particular al colectivo médico y a los pacientes. Las respuestas a este reto no pueden ser simples o unidimensionales, sino que implicarán múltiples factores y una mayor necesidad de coordinación principalmente entre la industria farmacéutica, los responsables de evaluar fármacos y los responsables de evaluar test genéticos.

La necesidad de adaptar el conocimiento y la práctica médica a los nuevos retos de la Farmacogenómica requiere cambios en la formación médica y una mayor implicación de los agentes reguladores traducida en provisión de guías clínicas asociadas al nuevo tipo de prescripción.

Asimismo, la Farmacogenómica supone un cambio en los fundamentos actuales de los sistemas sanitarios desde un enfoque reactivo a otro predictivo.