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Development and validation of a next-generation sequencing panel for clinical pharmacogenetics

Desarrollo y validación de un panel de secuenciación masiva en paralelo para farmacogenética clínica

Luis Ramudo-Cela[1,2], Jesús María López-Martí[1], Daniel Colmeiro-Echeberría[1], David de-Uña-Iglesias[1,2], José Luis Santomé-Collazo[1], Lorenzo Monserrat-Iglesias[1,2,3]

[1]Health in Code S. L., Science Department, A Coruña. Spain. [2] Biomedical Research Institute, A Coruña. Spain. [3]Universidade da Coruña, GRINCAR (Cardiovascular Research Group), A Coruña. Spain.

Author of correspondence
Luis Ramudo Cela
Edificio o Fortín
Hospital marítimo de Oza,
As Xuvias s/n
15006 A Coruña, Spain.

Email:
luis.ramudo@healthincode.com

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Abstract

The rapid clinical implementation of next generation sequencing techniques is due to its ability to sequence a large number of genetic regions at lower costs than conventional techniques. However, its use in the field of pharmacogenetics is still very limited.

Objective: Design, development, implementation and validation of a clinical pharmacogenetics next-generation sequencing panel.

Method: We developed a panel of hybrid capture probes (SureSelect®) for the analysis of the genetic regions of clinical interest collected by literature search and using Illumina HiSeq 1500® sequencing platform. We developed a bioinformatic algorithm for variant annotation, haplotype inference and determination of structural variants in the genes of interest. The results obtained were validated with Coriell® reference material from the pharmacogenetic repositories.

Results: The developed panel allows the study of a total of 12,794 regions comprised in 389 genes. Validation results showed a sensitivity greater than 99% for single nucleotide variants and small INDELs. Haplotype imputation was consistent with the consensus results in the characterized reference materials. Furthermore, the developed tool was able to correctly identify different types of CYP2D6 copy number variations as well as a wide variety of HLA-B alleles.

KEYWORDS

High-throughput nucleotide sequencing; Pharmacogenetics; Precision medicine; Computational biology; CYP2D6; HLA-B; DNA copy number variations.

Resumen

La rápida implantación clínica de las técnicas de secuenciación masiva en paralelo se debe a su capacidad para secuenciar un gran número de regiones genéticas con un coste menor a las técnicas convencionales. Sin embargo, su uso en el ámbito de la farmacogenética es, todavía, muy escaso.

Objetivo: Diseño, desarrollo, implementación y validación de un panel de secuenciación masiva en paralelo de farmacogenética orientado a la práctica clínica.

Método: Se desarrolló un panel de sondas de captura híbrida (SureSelect®) para el análisis de las regiones genéticas de interés clínico recopiladas mediante búsqueda bibliográfica. Se empleó la plataforma de secuenciación Illumina HiSeq 1500®. Se desarrolló un algoritmo de análisis bioinformático para la anotación de variantes puntuales, inferencia de haplotipos y determinación de variantes estructurales en los genes de interés. Los resultados obtenidos se validaron con materiales de referencia Coriell® de los repositorios de farmacogenética.

Resultados: El panel desarrollado permite el estudio de un total de 12.794 regiones comprendidas en 389 genes. Los resultados de validación mostraron una sensibilidad superior al 99% para variantes puntuales e inserciones y deleciones pequeñas. La imputación de haplotipos fue coherente con los resultados consenso de los materiales de referencia caracte-

PALABRAS CLAVE

Secuenciación de alto rendimiento; Farmacogenética; Medicina de precisión; Biología computacional; CYP2D6; HLA-B; Variaciones del número de copias de ADN.

Conclusions: This technology represents an appropriate alternative for its clinical use with advantages over conventional techniques in its throughput and complex gene study capabilities (CYP2D6, HLA-B).

rizados. Además, la herramienta desarrollada pudo identificar correctamente diferentes tipos de variaciones de número de copias de CYP2D6, así como una gran variedad de alelos de HLA-B.

Conclusiones: Esta tecnología representa una alternativa adecuada para su empleo asistencial con ventajas frente a las técnicas convencionales en su rendimiento de producción y sus capacidades de estudio de genes complejos (CYP2D6, HLA-B).

Introduction

Pharmacogenetics is the study of the influence of the genotype on the response to drugs. Clinical pharmacogenetics proposes individualized strategies for drug management based on each patient's genotype. Its final aim is to improve the healthcare outcomes of pharmacological treatments, improve efficacy, reduce adverse effects, and improve cost-benefit ratios: that is, it is a strategy for the rational use of drugs[1].

The incorporation of genetic data into healthcare processes requires high-quality sequencing technology, as well as standardized analysis and interpretation processes. In recent years, there has been a disruptive technological leap due to the development of massively parallel sequencing techniques or next-generation sequencing (NGS). Before this leap, only dozens of variants could be studied, but now tens of thousands can be studied. NGS is increasingly being incorporated into medical diagnostic processes. However, within the field of pharmacogenetics, conventional technologies such as quantitative PCR or primer extension methods such as TaqMan® (Thermofisher®) or MassARRAY® (Sequenom®) continue to be used[2,3].

These conventional technologies do not allow the in-depth study of all genetic regions of interest and can only examine subsets of them. Several reviews and comparative studies have addressed pharmacogenetic techniques, finding differences between different laboratories and technologies in the analysis and interpretation of results. It has been suggested that this result is due to the fact that different laboratories analyse different genetic regions without including the entire spectrum of variants of interest[4-6]. This aspect reduces the clinical value of the studies, creates mistrust in the results, and delays the incorporation of high-quality pharmacogenetics into clinical care practice.

This article presents the design, development, implementation, and validation of a pharmacogenetics NGS sequencing panel oriented towards clinical practice.

Methods

Definition of genetic regions of clinical interest

A literature search was conducted to select regions of interest. A region was considered clinically relevant if it provided information that could modify the therapeutic strategy relative to a given drug (e.g. treatment selection, dosage, patient follow-up). The main literature sources used were the clinical practice guidelines of scientific pharmacogenetic societies, technical files from drug regulatory agencies, and databases related to genetics (see Table 1). The main conventional pharmacogenetic platforms reviewed were Affymetrix DMET® and Agena Bioscience iPLEX®. Some genetic regions, such as CYP2D6 or HLA-B, required a specific design. In order to study copy number variations (CNVs) and structural variants of CYP2D6, probes were designed to capture all coding regions and regions of high homology (CYP2D7 and CYP2D8). For HLA-B, probes were designed against the reference sequences of the IMGT/HLA database[7]. The approach used was similar to that of Wittig *et al.*[8].

Capture probe design and sequencing process

Hybrid capture probes for these genetic regions were designed using SureSelect® Design Tool (Agilent) software. An automated genomic DNA extraction process (Qiasymphony SP®, Qiagen®) was conducted on the

Table 1. Information sources

Classification	Source		URL
Scientific Organisation Responsible for Clinical Practice Guidelines	CPIC	Clinical Pharmacogenetis Implentation Consortium	https://cpicpgx.org/
	DPWG	Dutch Pharmacogenetics Working Group	https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica/pharmacogenetics-1
	CPNDS	Canadian Pharmacogenetics Group for Drug Safety	http://cpnds.ubc.ca/
Drug Regulation Agencies	FDA	U. S. Food and Drug Administration	https://www.fda.gov/
	EMA	European Medicines Agency	https://www.ema.europa.eu/en
	AEMPS	Agencia Española de Medicamentos y Productos Sanitarios	https://www.aemps.gob.es/
Databases on pharmacogenetics, medical genetics, and population genetics	PharmGKB	Pharmacogenomics Knowledge Base	https://www.pharmgkb.org/
	PharmVar	Pharmacogene Variation Consortium	https://www.pharmvar.org/
	PharmaADME		http://www.pharmaadme.org/joomla/
	ClinVar		https://www.ncbi.nlm.nih.gov/clinvar/
	dbSNP	Single Nucleotide Polymorphism Database	https://www.ncbi.nlm.nih.gov/snp/
	gnomAD	Genome Aggregation Database	https://gnomad.broadinstitute.org/
	EVS	Exome Variant Server	http://evs.gs.washington.edu/EVS/
Bioinformatic predictors of the effects of mutations on proteins	SIFT	Sorting Intolerant From Tolerant	https://sift.bii.a-star.edu.sg/
	PMut	Pathogenic Mutation Prediction	http://mmb.pcb.ub.es/PMut/
	CADD	Combined Annotation Dependent Depletion	https://cadd.gs.washington.edu/

samples. Genomic DNA was prepared using the SureSelect XT® (Agilent Technologies®) protocol in combination with a SureSelect Custom Target Enrichment® probe panel, which selectively captures genomic regions of interest. Sequencing was done using Illumina HiSeq 1500® systems. All procedures were conducted following the manufacturer's specifications[9-11].

Data analysis and interpretation

We used an in-house bioinformatic analysis algorithm, which can annotate single nucleotide polymorphisms (SNPs) variants, Insertion-Deletion (INDELs), and structural variants (i.e. CNVs). CNVs were annotated by comparative analysis of read depth[12-14]. The variants identified were cross-referenced using an in-house knowledge management system. This system associates specific quality and information values to each variant using structural and SNP data from sources such as the Single Nucleotide Polymorphism Database (dbSNP), 1,000 genomes project[15], and the Exome Variant Server (EVS), as well as frequency data of the alleles in different populations, or values of bioinformatic predictors such as SIFT, PMut, and CADD (see table 1)[12-14]. Automated haplotype inference based on data from SNPs and small INDELs was performed using tables created in the knowledge management system based on the information sources (see table 1), especially PharmVar and PharmGKB. CYP2D6 haplotype inference was performed using a module that filters the alignments of CYP2D7 and CYP2D8. The aim of this process is to avoid artefacts in the coverage graphs of the reads in homologous regions[16]. This module also normalizes the read depth data using a control sample with a known and sequenced CYP2D6 copy number on the same assay plate. The aim of this normalization procedure is to avoid artefacts due to the presence of CNVs in other samples sequenced on the same plate[17] HLA-B allele were inferred using a specific module based on the work of Wittig *et al.*[8].

Analytical validation of results using reference materials

The determination of SNPs and small INDELs (< 20 bp) was validated using the Coriell® NA12878 sample. The sample was processed in triplicate and the results were compared to the reference data. This data was the result of integrating several datasets from entire-genome sequencing on different next-generation sequencing platforms[18]. We calculated the median values for analytical sensitivity (Se), specificity (Sp), and positive (PPV) and negative (NPV) predictive values. This analysis was conducted with three different quality filters using two NGS quality parameters: Quality Score (Qual) and read depth per sample (DP). The sequencing quality filters were defined as high when Qual was more than 49 and DP more than 29, intermediate when Qual was more than 49 and DP more than 14, and low when Qual was more than 0 and DP more than 0[19].

The determination of pharmacogenetic haplotypes based on SNPs and small INDELs was validated using the Coriell® sample NA12878. The results were compared to the reference data, which had been constructed by integrating various data sets from different pharmacogenetic platforms (GeT-RM project)[20,21]. These data include the following genes: *CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4, CYP3A5, CYP4F2, DPYD, GSTM1, GSTP1, GSTT1, NAT1, NAT2, SLC15A2, SLC22A2, SLCO1B1, SLCO2B1, TPMT, UGT1A1, UGT2B7, UGT2B15, UGT2B17, VKORC1*. Due to the complexity of *HLA-B* and *CYP2D6*, more probands were included: Coriell® samples NA12878, NA02016, NA17254, and NA17281 include different types of structural variants of CYP2D6 and HLA-B alleles of clinical interest (HLA-B*58:01)[8,20,21]. This analysis established concordance between the assayed haplotypes and the reference haplotypes.

Results

Genetic regions of interest included in the panel

The panel included a total of 12,794 base pairs distributed among 389 genes. The supplementary table 1 shows the size of the region under study for each gene and the average coverage of the region. These regions were classified into three groups: primary, secondary, and candidate genes according to the following internal criteria:

- Primary genes [17]: *CYP2C19, CYP2C9, CYP2D6, CYP3A5, CYP4F2, DPYD, F5, G6PD, HLA-B, IFNL3, RARG, SLC28A3, SLCO1B1, TPMT, UGT1A1, UGT1A6, VKORC1*. These genes are described in the main clinical practice guidelines drawn up by the main clinical pharmacogenetic consortia (see Table 1). They have the highest levels of evidence, and customized dosing strategies based on genotype have been published for all of them.
- Secondary genes [50]: *ABCB1, ABCC2, ABCG2, ACE, ADH1A, ADH1B, ADH1C, ADRB1, ADRB2, AHR, ALDH1A1, ALOX5, ASS1, COMT, CPS1, CYP1A1, CYP1A2, CYP2A6, CYP2C8, CYP2E1, CYP2J2, CYP3A4, DRD2, GSTM1, GSTP1, GSTT1, HMGCR, KCNJ11, MTHFR, NAT1, NAT2, NQO1, NR1I2, P2RY1, P2RY12, POLG, PTGIS, SCN1A, SLC15A2, SLC19A1, SLC22A1, SLC22A6, SLC6A4, SLCO1B3, SULT1A1, TYMS, UGT2B15, UGT2B17, UGT2B7, VDR*. Although secondary genes are not included in clinical practice guidelines, we consider that they may be of clinical interest according to the sources used (Table 1). This group includes the FDA lists of pharmacogenetic biomarkers, PharmGKB genes with levels of evidence 2 or higher, Clinical Pharmacogenetics Implementation Consortium (CPIC) priority categories A and B, or "core" genes from the pharmaADME list. Although no strategies for individualised treatment based on genotype have yet been published, we consider that the presence of variants in these genes may be useful for assessing the response to treatment in individual patients following a clinical finding.
- Candidate genes: these correspond to genomic regions with lower levels of evidence, but which are still included in one of the main pharmacogenomic platforms commonly used in clinical practice[22,23], or to regions described in the information sources (see Table 1), such as PharmGKB level 3 or 4, or CPIC categories C/D. These studies were set within an investigational framework. The supplementary material shows the full list of genes and regions.

Validation of the determination of SNPs and small INDELs

Table 2 shows the results of the determination of genetic variants of SNPs and INDELs of 20 bp or less. The analytical sensitivity and specificity of the assay were more than 99%. PPV and NPV were also more than 99% for all quality values.

Table 2. Validation of SNPs and INDELs < 20 bp

Sequencing quality	High	Medium	Low
True positives (TP)	553	572	573
False negatives (FN)	0	0	4
False positives (FP)	0	0	0
True negatives (TN)	12,241	12,222	12,217
Positive predictive value (PPV)	> 99.99%	> 99.99%	> 99.99%
Negative predictive value (NPV)	> 99.99%	> 99.99%	99.97%
Sensitivity (Se)	> 99.99%	> 99.99%	99.31%
Specificity (Sp)	> 99.99%	> 99.99%	> 99.99%

Note: Sample NA12878 was processed in triplicate and the results were compared with reference data generated on different mass sequencing platforms[18]. The median values for the three runs are shown. The sequencing quality filters were defined as high (Qual > 49 and DP > 29), intermediate (Qual > 49 and DP > 14), and low (Qual > 0 and DP > 0).

Validation of the determination of pharmacogenetic alleles (haplotypes)

Table 3 shows the results of the determination of pharmacogenetic haplotypes using the Coriell® NA12878 sample. Reference data is available for 28 genes each with 2 haplotypes (i.e. 56 haplotypes)[20,21]. The results were different for four genes: *CYP2D6, NAT2, SLCO1B1*, and *UGT2B17*. In the case of genes *CYP2D6, NAT2*, and *SLCO1B1*, the differences were due to the fact that the NGS platform examined more genetic regions, and thus improved the detection capabilities of the platforms used by the GeT-RM project[20,21]. Regarding CYP2D6, our NGS platform detected the presence of a tandem D6/D7 hybrid type rearrangement, which has been previously described[24,25]. In relation to *NAT2*, the technique was able to detect allele *12. This allele is only included in the Agena Bioscience iPLEX ADME PGx Pro® platform. In the case of *SLCO1B1*, our technique identified two possible combinations of haplotypes *1A/*15 and *1B/*5. This result is similar to that obtained using the Affimetrix DMET® platform and is due to the inclusion of the suballeles *1A and *1B. Our technique did not detect the presence of haplotype *2 in UGT2B17, which indicates a gene deletion. This requirement was not included in the design specifications. Overall, there was more than 98% concordance in the determination of alleles. Concordance would rise to 100% if the analysis is restricted to genes included in the main clinical practice guidelines (Table 1).

Validation of the results of the identification of CYP2D6 structural variants

Our technique was based on the comparative study of read depth, and was able to correctly identify the structural variants present in the four selected control samples. Figure 1 shows the results of the coverage

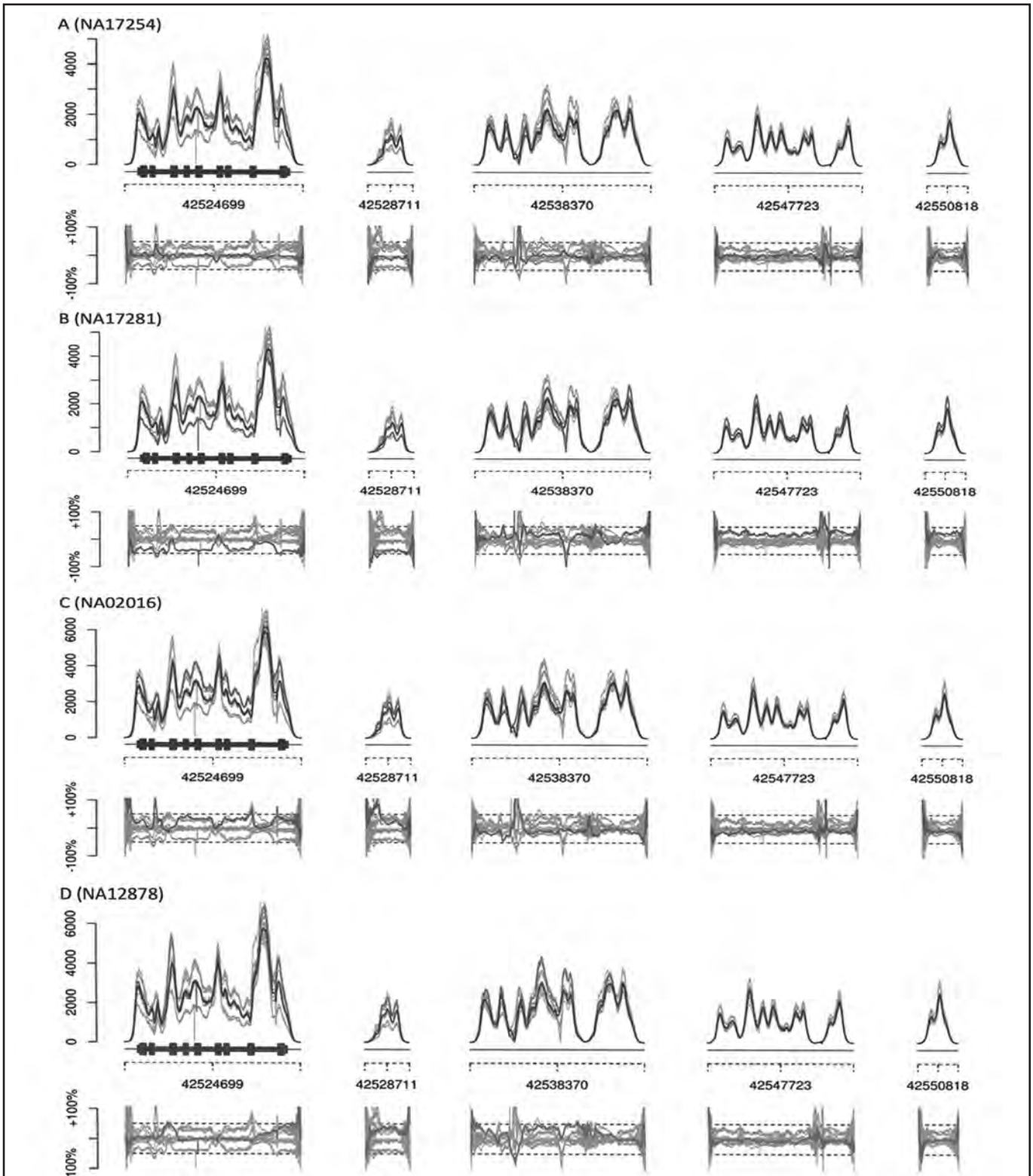
Table 3. Validation of the determination of pharmacogenetic alleles (haplotypes)

Gene	Reference alleles	Alleles identified by the technique
CYP1A1	*1/*1	*1/*1
CYP1A2	*1F/*1F	*1F/*1F
CYP2A6	*1/*1	*1/*1
CYP2B6	*1/*1	*1/*1
CYP2C8	*1/*3	*1/*3
CYP2C9	*1/*2	*1/*2
CYP2C19	*1/*2	*1/*2
CYP2D6	*3/*4	*3/*4+D6/D7 hybrid
CYP2E1 [1]	*5/*7	*5/*7
CYP3A4	*1/*1	*1/*1
CYP3A5	*3/*3	*3/*3
CYP4F2	*1/*1	*1/*1
DPYD	*1/*4	*1/*4
GSTM1	*B/*B	*B/*B
GSTP1 [2]	*A/*C;*B/*D	*A/*C;*B/*D
GSTT1	*A/*B	*A/*B
NAT1	*4/*4	*4/*4
NAT2	*4/*5	*12/*5
SLC15A2	*1/*1	*1/*1
SLC22A2	*1/*1	*1/*1
SLCO1B1	*1/*15	*1A/*15 o *1B/*5
SLCO2B1	WT / WT	*1/*1
TPMT	*1/*1	*1/*1
UGT1A1	*1/*28	*1/*28
UGT2B7	*1/*2	*1/*2
UGT2B15 [1]	*4/*4	*4/*4
UGT2B17	*2/*2	*1/*1
VKORC1 c.-1639G>A (rs9923231)	GA	GA

[1] GeT-RM results for sample NA12878 and CYP2E1 and UGT2B15 genes indicate "no consensus". This is due to the use of two different techniques: Affimetrix DMET® and Agena Bioscience iPLEX®. iPLEX® is unable to detect the CYP2E1*4 or UGT2B15*5 alleles. Therefore, the table includes the results from Affimetrix DMET®.
[2] The consensus result for GSTP1 is ambiguous: two possible allele combinations are established for a given combination of variants.

Farmacia Hospitalaria 2020
Vol. 44 N° 6 243 - 253

Figure 1. *Read depth and normalised read depth graphs.*



Note: Middle gray lines represent the depth of readings of the analyzed sample, darker gray lines represent the depth of readings of the control sample against which the depth of readings is normalized, and lighter gray lines represent the remaining samples analyzed on the same plate (grey lines). Read depth is represented for the genomic regions of CYP2D6 and the CYP2D7 and CYP2D8 pseudogenes. The samples are (A) NA17254 with CYP2D6*4/*41 genotype which is consistent two copies of CYP2D6, (B) NA17281 with CYP2D6*5/*9 genotype which is consistent with deletion in 1 of the alleles, (C) NA02016 with CYP2D6*2x2/*17 genotype, consistent with a duplication in 1 of the alleles, and (D) NA12878*3/*4+68 genotype, consistent with a tandem D6/D7 hybrid.

Table 4. Results of HLA-B allele identification

Coriell NA Sample	Canonical alleles	Alleles identified by the technique
NA17281	*39:06/*56:01	*39:06:02/*56:01:01
NA17254	*08:01/*35:01	*08:01:01/*35:01:01
NA02016	*53:01/*58:01	*53:01:01/*58:01:01
NA12878	*08:01/*56:01	*08:01:01/*56:01:01

graph analysis for CYP2D6. Sample NA17254 with the CYP2D6*4/*41 genotype presents two alleles of CYP2D6. This is the control sample against which the read depth of CYP2D6 is normalised. NA17281 with the CYP2D6*5/*9 genotype presents a deletion of one of the CYP2D6 alleles, and the normalized read depth decreased by approximately 50%. NA02016 with the CYP2D6*2x2/*17 genotype presents a duplication of one of the alleles, and had a 50% increase in read depth. Sample NA12878 with genotype*3/*4+68 presents a tandem D6/D7 hybrid, and had a 50% increase in read depth in part of the CYP2D6 region and part of the CYP2D7 region.

Validation of the results of the identification of HLA variants

The imputation technique developed to infer alleles in the HLA-B region was able to identify the alleles present in the three control samples. Table 4 shows the established consensus alleles and the results obtained using our technique.

Discussion

This article describes the development and analytical validation of a next generation sequencing platform for clinical pharmacogenetics, and presents the results obtained with the bioinformatics tools used to interpret the data.

Our panel includes a total of 12,794 base pairs distributed among 389 genes. The panel is much larger than that of conventional platforms: Affymetrix DMET® (1936 markers), Agena Bioscience® formerly (Sequenom®), iPLEX ADME PGx Pro® (270 markers), Illumina VeraCode ADME® (184 markers), and Roche Amplichip® (36 CYP2D6 and CYP2C19 alleles)[22,23].

The results from the control samples were consistent with the reference results of the GeT-RM project. Differences in the results were due to the higher capacity of our platform, except in the case of *UGT2B7*. For this gene, deletion-type structural variants have been described that would have required a specific design on our platform. However, this was not done because it has not been included in any of the main pharmacogenetic clinical practice guidelines.

It is difficult to study certain genomic regions of great pharmacogenetic interest, such as CYP2D6 and HLA-B. CYP2D6 metabolises approximately 25% of commercialized drugs[26] and its genotype is key to individualizing antidepressant treatments such as paroxetine[27], antipsychotics such as aripiprazole[28], tamoxifen[29], antiemetics such as ondansetron[30], or analgesics such as codeine[31], among others. It is difficult to study because of the existence of two regions with very high homology (i.e. CYP2D7 and CYP2D8), and the existence of CNV-like variants and pseudogene rearrangement (conversion-type and tandem hybrids with functional alleles). Other tools for the bioinformatic analysis of CYP2D6 have obtained good results in the analysis of SNP-type variants or small INDELs, but are of limited use in the determination of structural variants, especially hybrids[16,17,24,32,33]. This issue was addressed as described in the materials and methods section: our NGS technique filters the reads from the homologous regions and normalizes the read depth against a known control sample. The NGS platform appropriately identified different types of structural variants and CNVs analysed in the reference materials and provided graphs with the read depth showing the behaviour of coverage throughout the entire genomic region (see Figure 1).

The Human Leukocyte Antigen (*HLA*) region is the most densely polymorphic region of the genome. Its relevance in pharmacogenetics stems from its association with hypersensibility to immunomediated drugs[34]. Conventional typing techniques can only analyse a few haplotypes, while general procedures using NGS technologies do not yield good results due to variability and sequence homology between genes and pseudogenes[35]. The current NGS version incorporates a specific probe design and previously validated analysis software that enables the comprehensive study of HLA-B.

Compared to conventional platforms, the main limitations of the NGS platform are assay time, the need for qualified technicians, development and commissioning costs, and higher costs of data processing and storage on servers[36]. It takes approximately 10 days to obtain the results after a sample has been received (i.e. including sample preparation, sequencing, and bioinformatic analysis). These processes require technical staff with specific training and bioinformatics personnel to fine-tune the technique and analyse the sequencing data.

Although our technique and bioinformatics data process identified rare variants and other previously-undescribed variants, these results cannot be extrapolated to clinical care settings since there are no data in the literature for their interpretation. These rare variants account for over 80% of variations in the genes of interest in pharmacogenetics: however, conventional platforms are not oriented to their analysis[37,38]. These variants may be incorporated into clinical care practice when scientific articles on genotype-phenotype correlation data are published, consensus on interpretation is built, and protocols become available[39].

Finally, the evaluation and validation of this platform in prospective clinical studies would demonstrate the clinical value of this platform.

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Conflicts of interest

No conflict of interests.

Contribution to the scientific literature

This article describes the development, implementation, and validation of a next-generation sequencing panel for clinical pharmacogenetics.

Supplement table 1. Genomic regions, size, and average coverage by gene

Gene	Chromosome	Number regions	Total size (pb)	Mean coverage
A1BG	19	1	1	392.88
ABCB1	7	41	41	756.22
ABCB11	2	32	34	712.94
ABCB4	7	27	27	784.39
ABCB7	X	6	6	530.52
ABCC1	16	22	25	631.36
ABCC2	10	45	45	924.36
ABCC3	17	6	6	613.77
ABCC4	13	17	25	646.23
ABCC5	3	9	9	700.81
ABCC6	16	7	7	442.11
ABCC8	11	7	8	543.95
ABCC9	12	6	6	570.61
ABCG1	21	7	7	690.41
ABCG2	4	11	11	695.70
ACE	17	9	9	551.54
ADD1	4	1	1	578.25
ADH1A	4	7	7	483.02
ADH1B	4	6	6	865.02
ADH1C	4	5	5	739.55
ADH4	4	6	6	667.13
ADH5	4	5	5	460.75
ADH6	4	6	6	540.27
ADH7	4	5	5	723.88
ADORA2A	22	3	3	288.09
ADRA2A	10	1	1	194.25
ADRB1	10	2	2	166.63
ADRB2	5	9	9	799.66
AGTR1	3	2	2	599.69
AHR	7	6	6	369.71
AKAP9	7	3	3	648.50
ALB	4	7	7	593.29
ALDH1A1	9	6	76	721.56
ALDH2	12	6	6	592.00
ALDH3A1	17	5	5	379.78
ALDH3A2	17	7	7	757.22
ALOX5	10	1	1	570.63
AMPD1	1	1	1	372.13
ANKK1	11	5	5	421.98
AOC1	7	7	7	475.68
AOX1	2	7	7	777.09
APOA2	1	1	1	555.25
APOB	2	1	1	453.63
APOC3	11	3	3	544.67
APOE	19	2	2	166.57
ARG1	6	1	1	759.75
ARNT	1	7	7	618.32
ARSA	22	5	5	400.48
ASS1	9	2	2	718.32
ATIC	2	10	10	606.15
ATP7A	X	7	7	656.25
ATP7B	13	10	10	582.63
BCHE	3	11	11	644.37
BDKRB1	14	1	1	652.75
BDKRB2	14	2	2	394.44
C16orf54	16	1	1	512.13
C6orf10	6	1	1	460.00
C8orf34	8	1	1	709.25
CACNA1C	12	1	1	456.25
CACNA1S	1	2	2	541.63
CACNA2D1	7	1	1	395.38
CACNB2	10	2	2	488.32
CACNG2	22	1	1	987.63
CALU	7	3	3	595.33
CBR1	21	6	6	767.71
CBR3	21	6	6	635.67
CCDC170	6	1	1	608.00
CCDC174	3	7	7	654.90
CCDC33	15	7	7	630.07
CCHCR1	6	2	2	504.94
CDA	1	7	7	554.68
CDSN	6	1	1	0.38
CES1	16	13	13	737.25
CES2	16	7	7	478.79
CETP	16	8	8	607.27
CFTR	7	19	24	994.56
CHRNA3	15	3	3	531.46
CHRNA4	20	2	2	476.76
CHST1	11	6	6	607.48
CHST10	2	14	14	890.26
CHST11	12	7	7	684.70
CHST13	3	6	6	612.98
CHST2	3	5	5	537.85
CHST3	10	16	16	805.07
CHST4	16	3	3	442.42
CHST5	16	7	7	577.66
CHST6	16	3	3	725.29
CHST7	X	5	5	409.53
CHST8	19	3	3	523.96
CHST9	18	5	5	686.73
COL22A1	8	1	1	556.25
COMT	22	8	8	524.55
COQ2	4	2	2	476.07
CPS1	2	1	1	860.25
CRHR1	17	30	30	618.46
CRHR2	7	6	6	581.52
CRIP3	6	7	7	643.73
CROT	7	4	4	597.88

Supplement table 1 *(cont.).* Genomic regions, size, and average coverage by gene

Gene	Chromosome	Number regions	Total size (pb)	Mean coverage
CRYAB	11	1	1	257.13
CYB5R3	22	4	4	336.35
CYP11B1	8	17	17	641.75
CYP11B2	8	5	5	568.03
CYP17A1	10	6	6	881.71
CYP19A1	15	11	11	840.04
CYP1A1	15	13	13	882.79
CYP1A2	15	17	17	671.07
CYP1B1	2	21	106	886.47
CYP20A1	2	5	5	661.43
CYP21A2	6	3	3	280.46
CYP24A1	20	8	10	933.41
CYP26A1	10	5	5	560.98
CYP26C1	10	1	1	469.50
CYP27A1	2	5	5	710.73
CYP27B1	12	9	9	743.06
CYP2A13	19	5	5	701.98
CYP2A6	19	20	26	826.22
CYP2A7	19	12	12	813.69
CYP2B6	19	23	23	846.89
CYP2C18	10	7	7	672.18
CYP2C19	10	21	21	883.92
CYP2C8	10	12	23	781.26
CYP2C9	10	20	32	970.51
CYP2D6	22	115	4787	930.94
CYP2E1	10	10	10	593.28
CYP2F1	19	5	5	549.90
CYP2J2	1	9	9	767.27
CYP2S1	19	4	4	646.32
CYP39A1	6	4	4	631.63
CYP3A4	7	26	26	766.96
CYP3A43	7	7	7	554.39
CYP3A5	7	16	16	846.99
CYP3A7	7	7	7	968.25
CYP46A1	14	1	1	529.00
CYP4A11	1	4	4	497.44
CYP4B1	1	7	9	1038.11
CYP4F11	19	4	4	509.25
CYP4F12	19	6	6	709.50
CYP4F2	19	11	11	579.92
CYP4F3	19	14	14	584.74
CYP4Z1	1	4	4	433.35
CYP51A1	7	7	7	468.00
CYP7A1	8	7	7	455.56
CYP7B1	8	4	4	495.25
CYP8B1	3	7	7	563.56
DBH	9	1	1	412.38
DCK	4	4	4	554.01
DDTL	22	2	2	68.13
DPYD	1	18	31	834.48
DRD1	5	1	1	232.75
DRD2	11	25	25	584.49
DRD3	3	2	2	523.44
DRD4	11	1	1	100.50
DSG2	18	1	1	421.50
DTNBP1	6	2	2	468.82
DYNC2H1	11	1	1	614.88
DYRK1A	21	1	1	537.00
EDN1	6	1	1	928.50
EGF	4	8	8	557.36
EPHX1	1	13	13	781.79
EPHX2	8	5	8	523.70
ERCC1	19	3	3	488.42
ERCC6L2	9	1	1	649.75
ESR2	14	1	1	287.38
F2	11	1	1	451.25
F5	1	3	3	590.88
F7	13	1	1	390.38
FAAH	1	2	2	585.19
FCGR3A	1	1	1	408.88
FDPS	1	1	1	527.88
FKBP5	6	5	5	444.65
FLOT1	6	2	2	341.75
FMO1	1	28	43	797.37
FMO3	1	31	31	1009.32
FMO4	1	6	6	790.82
FMO5	1	7	11	756.06
FTO	16	1	1	854.50
G6PD	X	204	255	1164.76
GABRA6	5	1	1	478.75
GALC	14	1	1	555.50
GGCX	2	5	13	561.63
GLCCI1	7	1	1	456.00
GNB3	12	2	2	432.13
GOLGA7	8	1	21	35.00
GP1BA	17	2	2	185.32
GRIK4	11	2	2	668.63
GRK4	4	3	3	802.59
GRK5	10	1	1	0.00
GSTA1	6	12	12	772.23
GSTA2	6	7	7	783.13
GSTA3	6	7	7	826.06
GSTA4	6	7	7	676.77
GSTA5	6	7	7	670.57
GSTM1	1	3	3	115.50
GSTM2	1	5	5	837.38
GSTM3	1	6	6	730.84
GSTM4	1	3	3	1207.38

Supplement table 1 ***(cont.).*** Genomic regions, size, and average coverage by gene

Gene	Chromosome	Number regions	Total size (pb)	Mean coverage
GSTM5	1	5	5	712.23
GSTO1	10	5	5	748.93
GSTP1	11	5	5	433.15
GSTT1	22	6	6	386.80
GSTZ1	14	4	4	544.97
HAS3	16	1	1	271.75
HLA-A	6	1	1	931.25
HLA-B	6	53	3743	1061.70
HLA-C	6	1	1	502.38
HLA-DQA1	6	1	1	428.75
HLA-DRB1	6	1	1	320.63
HLA-F	6	1	1	943.00
HMGCR	5	9	9	480.38
HNMT	2	6	10	453.82
HTR1A	5	5	5	505.13
HTR2A	13	10	10	591.97
HTR2C	X	7	7	371.68
IFNL3	19	7	7	409.30
IFNL4	19	5	6	452.73
IL1B	2	1	1	536.75
IMPDH1	7	2	2	269.51
ITGB3	17	1	1	514.13
ITPA	20	1	1	715.50
KCNIP1	5	2	2	506.07
KCNIP4	4	6	6	430.36
KCNJ11	11	1	1	346.13
KCNMB1	5	2	2	506.07
KIF6	6	3	3	661.13
LDLR	19	11	11	473.49
LEP	7	2	2	573.44
LPA	6	2	2	679.82
LPIN1	2	1	1	665.38
LTA	6	2	2	732.13
LTA4H	12	1	1	560.88
LTC4S	5	1	1	289.50
LUC7L2	7	1	1	663.88
LYZ	12	1	1	349.25
MAOB	X	13	13	584.88
MAT1A	10	7	7	643.02
MC4R	18	4	4	693.63
MED12L	3	1	1	399.00
METTL1	12	1	1	615.75
MICA	6	1	1	408.00
MICB	6	1	1	557.38
MTHFR	1	6	6	668.63
MTRR	5	3	3	686.63
MUC21	6	1	1	616.50
MYH7	14	1	1	252.25
MYLIP	6	1	1	832.50
NAT1	8	21	29	942.85
NAT2	8	16	16	1031.74
NEDD4L	18	5	5	602.75
NELFCD	20	1	1	753.25
NNMT	11	7	8	816.15
NOS3	7	2	2	456.26
NPPA	1	1	1	760.88
NQO1	16	7	7	669.34
NR1I2	3	16	16	778.78
NR1I3	1	6	6	803.19
NR3C1	5	12	12	702.65
NT5C2	10	2	2	474.94
NUDT15	13	2	2	667.82
OPRD1	1	1	1	493.38
OPRK1	8	2	2	241.88
OPRM1	6	8	8	506.55
ORM1	9	4	4	1132.94
ORM2	9	2	2	745.63
P2RY1	3	2	2	464.44
P2RY12	3	1	1	399.00
PARP12	7	25	25	878.85
PCSK9	1	1	1	298.63
PGAP3	17	1	1	279.63
PLEC	8	1	1	262.63
PNMT	17	3	3	211.88
POLG	15	1	1	540.63
PON1	7	5	8	793.98
PON2	7	4	4	500.51
PON3	7	7	7	908.86
POR	7	3	3	631.96
PPARD	6	47	47	607.97
PPARG	3	6	6	738.11
PPP1R9A	7	1	1	283.88
PRKCA	17	2	2	661.88
PROC	2	3	3	650.09
PROS1	3	1	1	481.75
PRSS53	16	1	1	354.50
PSORS1C1	6	1	1	0.38
PTGFR	1	2	2	660.07
PTGIS	20	12	12	592.26
PTGS1	9	8	8	597.71
QPRT	16	1	1	519.25
RALBP1	18	6	6	465.84
RARG	12	1	1	262.88
RGS4	1	3	3	490.13
RPF2	6	1	1	732.38
RPL13	16	2	2	395.57
RPP21	6	1	1	0.00
RXRA	9	4	4	426.91

Supplement table 1 *(cont.).* Genomic regions, size, and average coverage by gene

Gene	Chromosome	Number regions	Total size (pb)	Mean coverage
RYR1	19	45	45	674.46
SCN1A	2	3	3	561.75
SELE	1	1	1	952.38
SELP	1	1	1	507.63
SEMA3C	7	2	2	769.50
SERPINA7	X	7	7	763.02
SERPINE1	7	1	1	367.25
SIGLEC12	19	1	1	638.50
SIVA1	14	1	1	269.00
SLC10A1	14	7	7	978.81
SLC10A2	13	8	8	711.30
SLC13A1	7	7	7	580.45
SLC14A2	18	2	2	735.07
SLC15A1	13	19	19	812.04
SLC15A2	3	10	10	906.10
SLC16A1	1	7	7	836.32
SLC19A1	21	5	5	438.13
SLC22A1	6	30	31	942.09
SLC22A11	11	6	6	510.84
SLC22A12	11	2	2	403.07
SLC22A13	3	7	7	507.05
SLC22A14	3	6	6	588.15
SLC22A16	6	1	1	519.13
SLC22A2	6	16	19	752.27
SLC22A3	6	5	5	685.65
SLC22A4	5	7	16	674.49
SLC22A5	5	8	8	717.52
SLC22A6	11	7	8	447.47
SLC22A8	11	11	11	636.59
SLC25A27	6	3	3	410.25
SLC28A1	15	27	27	794.69
SLC28A2	15	7	7	950.50
SLC28A3	9	7	7	644.59
SLC29A2	11	6	6	484.54
SLC35B2	6	9	9	693.46
SLC47A2	17	3	3	338.55
SLC5A6	2	7	7	597.11
SLC6A4	17	7	46	255.18
SLC7A5	16	3	3	700.17
SLC7A7	14	7	7	685.41
SLC7A8	14	7	7	605.40
SLC9A7	X	2	2	383.75
SLCO1A2	12	16	17	540.64
SLCO1B1	12	18	18	643.56
SLCO1B3	12	7	11	556.13
SLCO2B1	11	7	7	648.79
SLCO3A1	15	6	7	572.52
SLCO4A1	20	5	5	521.43
SLCO5A1	8	5	5	482.43
SOD2	6	2	2	548.19
SPECC1L	22	1	1	200.13
SPG7	16	8	8	620.44
SPN	16	7	7	373.72
SRY	Y	7	715	40.86
SULT1A1	16	3	3	678.67
SULT1A2	16	5	5	587.78
SULT1B1	4	6	6	514.31
SULT1C2	2	9	9	796.60
SULT1C4	2	7	7	663.61
SULT1E1	4	5	5	607.40
SULT2A1	19	6	6	727.44
SULT2B1	19	7	7	618.47
SULT4A1	22	5	5	566.50
TANC1	2	7	7	721.70
TBL1Y	Y	1	1	125.75
TCF7L2	10	7	7	604.73
TGFBR3	1	1	1	97.50
TNF	6	9	9	904.04
TOMM40	19	1	1	516.88
TPH2	12	2	2	627.69
TPMT	6	9	9	554.04
TPSG1	16	2	2	231.07
TRIM40	6	1	1	0.13
TSPY8	Y	1	1	122.00
TTN	2	1	1	347.50
TYMS	18	5	5	1027.90
UGT1A1	2	29	62	1074.66
UGT2A1	4	7	7	622.09
UGT2B11	4	3	3	780.13
UGT2B15	4	6	6	616.94
UGT2B17	4	14	504	147.66
UGT2B28	4	3	3	686.79
UGT2B4	4	7	7	631.29
UGT2B7	4	8	8	739.52
UGT8	4	5	5	1058.18
UMPS	3	4	4	726.35
USP24	1	1	1	752.50
USP40	2	8	8	1044.28
USP9Y	Y	1	1	110.88
VDR	12	3	3	593.46
VKORC1	16	22	22	613.28
XDH	2	8	8	666.47
XRCC1	19	3	3	468.79
YEATS4	12	1	1	535.13
ZBTB42	14	1	1	623.38
ZNRD1	6	1	1	0.00

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