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Deliverable 2.1
List of relevant genetic variants for pre-emptive PGx testing

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Comment:

Introduction

In recent years a number of scientific studies have shown that genetic variation in genes encoding drug transporters and drug metabolic enzymes affects drug disposition leading to under-dosing or overdosing in affected individuals. Furthermore, variation in genes encoding drug targets has also been shown to have a major influence on a drug's effect, influencing both efficacy and safety. Pharmacogenomics (PGx) testing prior to prescribing leads to improved patient outcome.

Based upon the PGx guidelines a uniform panel of clinically relevant genetic variants in following genes *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, dihydropyrimidine dehydrogenase (*DPYD*), *F5*, major histocompatibility complex, class I (*HLA-A and HLA-B*), solute carrier organic anion transporter family member 1B1 (*SLCO1B1*), thiopurine-S-methyltransferase (*TPMT*), uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) and vitamin K epoxide reductase (*VKORC1*) is selected. The objective is that if these variants are determined in clinical practice, it is cost-effective and results in a better outcome for patients.

PGx variants in 13 genes were selected. The criteria for inclusion were:

- Is there a DPWG guideline with therapeutic recommendations for the specific genotype?
- Is the effect of the variant on the gene expression established? (e.g. Is CYP2C9 inactive if the variant is present?)
- Is the overall MAF[1] ≥ 1%?
  - If not, is the MAF in selected populations (European/Asian/African)[2] ≥ 1%?
- If the MAF is below 1% in all cases, selection of certain variants is possible if at least one of the implementation sites already determines the allele in patient care.

1. MAF = minor allele frequency. For the determination of the MAF we used www.ensembl.org and received input from Karolinksa Institutet and the DPWG. A MAF of 1% or greater is considered to be common. We restricted allele selection above this selected MAF cut off.
2. The selected populations are the most common populations in Europe. Besides the Europeans, Asians and Africans are present in Europe due to migration.

Results

Based on the selection criteria 3 variants in *CYP2B6*, 4 in *CYP2C9*, 9 in *CYP2C19*, 11 in *CYP2D6*, 3 in *CYP3A5*, 4 in *DPYD*, 1 in *F5*, 1 in *HLA-A,* 2 in *HLA-B*, 1 in *SLCO1B1*, 3 in *TPMT*, 3 in *UGT1A1*, and 1 in *VKORC1* were selected. We highlight variants that have been discussed or variants included based on the last criterion (already used in patient care).

In order to distinguish *CYP2C19*4* and *17 both variants 1A>G and -806C>T were included,

although in 1A>G the overall MAF and the MAF in the selected populations are <1%. Haplotypes *5, *6, *7, *8 and *10 were selected based on the patient care criterion (these variants are present in standard genotyping platforms). *CYP2C19*19* can only be determined with variants 87106T>C and 151A>G. Since variant 87106T>C is also present in *18, determination of *19 is not possible with only 87106T>C. However, both variants were excluded from the selection, since the functionality of *CYP2C19*19* is only shown in vitro in recombinant studies.

In *CYP2D6* haplotypes *xN and *5 were selected because they are used in patient care. For *xN there is no MAF since this represents multiple copies of the same variant, but CPIC indicates a frequency ≥ 1% in patients. Variants 100C>T and 1758G>A (rs5030865) are necessary to distinguish haplotypes*10 and *14A/B. On the other hand, rs5030865 is a triallelic variant (G>A/T) and defines with allele T haplotype *8 as well. For this reason *8 is also included, although the overall MAF and the MAF in the selected populations are <1%. Variant 2850C>T in *17 was excluded, since 1023C>T is the detrimental mutation and is sufficient for identification of *17. In *29 variant 3183G>A is the crucial mutation and is sufficient for identification of *29. Due to haplotype issues both 1659G>A and 3183G>A were included.
A number of *CYP2D6* variants met the criteria for the MAF, but there were not specific for haplotypes with an established effect or expert opinion indicated the real MAF was below the threshold. These alleles were excluded.

Besides 1236G>A (MAF ≥ 1%), the haplotypes in *DPYD* were selected because at least one of the implementation sites already determines the allele in patient care.

TPMT allele *2 is selected, since at least one of the implementation sites already determines the allele in patient care. Allele *3B (460G>A), *3C (719A>G) and *3A (both variants 460G>A and 719A>G) will be evaluated with software to determine the haplotype.

In *UGT1A1*, allele *28 and *37 both result in decreased UGT1A1 activity. Due to the high sequence complexity it will not be possible to develop specific
assays, so both variants are merged.

The *VKORC1* variants -1639G>A and 1173 C>T are in strong linkage disequilibrium. Determination of both variants rarely provides more information than determination of only one of them. Therefore, we only selected variant 1173 C>T.

CYP1A2 and *CYP3A4* alleles were not included, since the available DPWG guidelines has no therapeutic recommendations. The selected genes and respective variants are listed in table 1.

Table 1: Selected pharmacogenes and respective variants (RS number included).

Genes	Allele	Major Nucleotide Variation	dbSNP RS ID	Effect on protein	Functional Status
CYP2B6	*6/*9	516G>T	rs3745274	Q172H	Decreased or Inactive
CYP2B6	*4/*16	785A>G	rs2279343	K262R	Decreased or Inactive
CYP2B6	*18	983T>C	rs28399499	I328T	Decreased or Inactive
CYP2C9	*2	430C>T	rs1799853	R144C	Decreased
CYP2C9	*3	1075A>C	rs1057910	I359L	Decreased
CYP2C9	*5	1080C>G	rs28371686	D360E	Decreased
CYP2C9	*11	1003C>T	rs28371685	R335W	Decreased
CYP2C19	*2	681G>A	rs4244285	Splicing defect	Inactive
CYP2C19	*3	636G>A	rs4986893	W212X	Inactive
CYP2C19	*4A/B	1A>G	rs28399504	M1V	Inactive
CYP2C19	*5	1297C>T	rs56337013	R433W	Inactive
CYP2C19	*6	395G>A	rs72552267	R132Q	Inactive
CYP2C19	*8	358T>C	rs41291556	W120R	Inactive or Decreased
CYP2C19	*9	431G>A	rs17884712	R144H	Decreased
CYP2C19	*10	680C>T	rs6413438	P227L	Decreased
CYP2C19	*17	-806C>T[3]	rs12248560	X	Increased
CYP2D6	*xN	Gene duplication or multiplication	X	X	Increased
CYP2D6	*3	2549delA	rs35742686	259Frameshift	Inactive
CYP2D6	*4	1846G>A	rs3892097	Splicing defect	Inactive
CYP2D6	*5	Gene deletion	X	Gene deletion	Inactive
CYP2D6	*6	1707delT	rs5030655	118Frameshift	Inactive
CYP2D6	*8	1758G>T	rs5030865	G169X	Inactive
CYP2D6	*9	2615delAAG	rs5030656	K281 deletion	Decreased
CYP2D6	*10	100C>T	rs1065852	P34S	Decreased
CYP2D6	*14A/B	1758G>A	rs5030865	G169R	Decreased
CYP2D6	*17	1023C>T	rs28371706	T107I	Decreased
CYP2D6	*41	2988G>A	rs28371725	Splicing	Decreased
CYP3A5	*3	6986A>G	rs776746	Splicing defect	Inactive
CYP3A5	*6	14690G>A	rs10264272	Splicing defect	Inactive
CYP3A5	*7	27131_27132insT	rs41303343	346Frameshift	Inactive
DPYD	*2A	IVS14 + 1G>A (1905+1G>A)	rs3918290	X	Inactive
DPYD	*13	1679T>G	rs55886062	I560S	Inactive
DPYD	X	2846A>T	rs67376798	D949V	Decreased
DPYD	X	1236G>A	rs56038477	E412E	Decreased
F5	X	1691G>A	rs6025	R506Q	Decreased
HLA-B	*5701	T>G	rs2395029		Tagging SNP
HLA-B	*1502	G>C	rs3909184		Tagging SNP
HLA-B	*1502	G>A	rs2844682		Tagging SNP
HLA-A	*3101	T>C	rs1633021		Tagging SNP Asian

HLA-A	***3101**	A>T	rs1061235		Tagging SNP Caucasian
SLCO1B1	***5/*15/*17**	521T>C	rs4149056	V174A	Decreased
TPMT	***2**	238G>C	rs1800462	A80P	Inactive
TPMT	***3B**	460G>A	rs1800460	A154T	Inactive
TPMT	***3C**	719A>G	rs1142345	Y240C	Inactive
UGT1A1	***6**	211(G>A)	rs4148323	G71R	Decreased
UGT1A1	***27**	686(C>A)	rs35350960	P229Q	Decreased
UGT1A1	***28/*37**	A(TA)6TAA>A(TA)7 TAA/A(TA)8TAA	rs8175347	X	Decreased
VKORC1	**X**	1173C>T (C6484T)	rs9934438		Increased sensitivity

[3] Position in genomic DNA sequence is used, since there is no cDNA position for this mutation.

Note: this list is dynamic. Other genetic variants can be included during the project if a therapeutic recommendation is published for these variants.

Summary/Conclusions

A panel of 48 clinically relevant genetic SNPs (to determine 46 variants) is selected comprising *CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, F5, HLA-A, HLA-B, SLCO1B1, TPMT, UGT1A1* and *VKORC1*.

The criteria for inclusion were: (i) there is a DPWG guideline with therapeutic recommendations available; and (ii) the effect of the variant on the expression of the gene is established; and (iii) the overall MAF ≥ 1%, or the MAF in selected populations (European/Asian/African) ≥ 1%, or at least one of the implementation sites already determines the allele in patient care.