KNMP

Uncertainties in genotyping results

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***1-allele (wildtype allele)**
In practice, genotyping tests for the most common gene variations that result in altered activity. If an allele does not contain the tested gene variations, then this allele is called the wildtype allele (*1). However, as not all gene variations are tested, one can never be entirely certain that this allele does not contain a (non-tested) gene variation and is in fact not the wildtype allele (*1) and does not have normal activity.
However, if the most common gene variations, which result in altered activity, have been tested, then the chance of a *1 allele actually not being a *1 allele is small (only a few percent). Therefore, the recommendation is that the patient should always be linked to the pharmacogenetic indication based on the result once the most important gene variations have been tested. Deviations should only be considered if the most important gene variations in the population to which the patient belongs have not been tested.
NB: As the wildtype allele (*1) is generally also the most common allele, an incorrect classification of an allele as wildtype allele (*1) generally means that the patient will be treated as he or she would be treated if he or she had not been genotyped. The CYP3A5 gene forms an exception to this rule, as the *3 allele, which results in an inactive enzyme, is by far the most common allele in most population groups and *1 occurs only rarely. In this case, an incorrect *1 result would lead to the patient receiving different treatment than if he or she had not been genotyped. However, the chance of an incorrect *1 result is once again low, as the most important gene variations/alleles have been determined. If the *5 allele has not been determined for CYP3A5, then the chance of a reported genotype *1/*3 actually being *3/*5 is only 3-4% in Asians (*5 allele frequency 0.9%) and approximately 0% in people of another ethnicity (*5 allele frequency approximately 0%). Even if only *3 is determined (and therefore only 48% of the alleles that result in an inactive enzyme have been determined) in Africans (*1 frequency 47.9%, *3 frequency 24.1%, *6 frequency 19.3% and *7 frequency 8.6%), then the chance of a *1/*3 genotype actually being a *1/*3 genotype is still 63%. However, the 37% chance of the genotype not being *1/*3 in this case is high and most laboratories therefore perform determination of at least *3 and *6.

Alleles that result in an enzyme with reduced activity
In practice, genotyping tests are performed for the most common gene variations that result in altered activity, meaning that gene variations can also be missed for alleles other than the *1 allele. For alleles that result in an enzyme with reduced activity, an extra gene variation can result in an inactive enzyme instead of an enzyme with reduced activity. Most alleles are very well defined and this situation only occurs for a few allele combinations. For example, for CYP2D6, the characteristic gene variation in allele *14 can occur on its own - resulting in an allele with reduced activity - or in combination with another gene variation resulting in an inactive allele (*114).
However, if the most common gene variations, which result in altered activity, have been tested, then the chance of an allele with reduced activity actually being an inactive allele is small (only a few percent). Therefore, the recommendation is that the patient should always be linked to the pharmacogenetic indication based on the result once the most important gene variations have been tested. Deviations should only be considered if the most important gene variations for the ethnicity in question have not been tested.

Alleles that result in an inactive enzyme
There are no known examples in which a second gene variation eliminates or reduces the effect of a gene variation that results in an inactive enzyme. If the result points to an inactive allele, then this is usually the case (unless errors have been made in the genotyping).

Alleles and gene duplications that result in increased enzyme activity
The CYP2C19*17 allele results in increased enzyme activity as a result of increased enzyme production. This effect would be negated by an extra inactivating gene variation. At the moment there is only one known allele that has an inactivating gene variation in addition to the characteristic gene variation in the *17 allele: one of the two *4 alleles. As the frequency of CYP2C19*4 is much lower than that of CYP2C19*17 (0.6% versus 18-27% in Caucasians, 0.2% versus 1-4% in Asians and 0% versus 18% in Africans), the chance that a *17 result is not actually *17 but instead *4 is low, even if *4 has not been determined.
In the case of CYP2D6, the production of the enzyme is increased in the case of a gene duplication (or gene multiplication). A gene duplication will have no effect in the case of an inactive allele and only a limited effect on enzyme activity in the case of an allele with reduced activity. However, if the most common gene variations, which

result in altered activity, have been tested, then the chance of a *1 or *2 allele actually not being a *1 or *2 allele (so an allele without normal activity) is small (only a few percent).
Therefore, the recommendation is that the patient should always be linked to the pharmacogenetic indication based on the result once the most important gene variations have been tested. Deviations should only be considered if the most important gene variations in the population to which the patient belongs have not been tested.
NB: A problem with gene duplications (or gene multiplications) in patients with two alleles that differ in activity is that most laboratories do not determine which of the two alleles has been multiplied or how many copies the multiplication has resulted in. This means, for example, that the laboratories cannot comment on whether a combination of an inactive allele and a fully active allele and a gene multiplication would result in an intermediate metaboliser (multiplication of the inactive allele), a normal metaboliser (duplication of the fully active allele) or an ultra-rapid metaboliser (3 or more copies of the fully active allele). In these cases, the laboratory should indicate that the genotype and therefore also the resulting predicted phenotype could not be determined with certainty.

HLA alleles
In general, HLA alleles are genotyped by means of sequencing (determination of the complete base sequence of the variable part). This means that the outcome does not contain any uncertainty.
In some cases, gene variations located in nearby genes or DNA are used (tagging SNPs). As the relationship between these tagging SNPs and a certain HLA alleles is never 100% certain and can differ between various population groups, there is some degree of uncertainty about the result in these cases. The degree of uncertainty depends on extent of correlation between the tagging SNPs and the HLA allele in question in the various population groups. In general, laboratories will only use tagging SNPs in the case of a good correlation. However, the general background text for HLA includes a warning stating that there appears to be insufficient evidence of a good correlation for the commonly used tagging SNPs for HLA-B*1502, resulting in the recommendation by the KNMP Pharmacogenetics Working Party to ignore any results obtained with these tagging SNPs.

Gene variations determined instead of alleles
An allele can contain several gene variations. However, when genotyping is performed on some genes, the individual gene variations are determined instead of the alleles. Examples include VKORC1 -1639G>A, ABCG2 141Q>K, factor V Leiden and MTHFR 677C>T. DPYD (*2A, *13, 1236G>A and 2846A>T) is another example of this. Although the notations *2A and *13 for DPYD appear to refer to alleles, they actually refer to gene variations. As the alleles for DPYD are poorly characterised, it is not known which gene variations can occur together on an allele and thus which alleles exist. In other words, only gene variations can be determined.
Genotyping provides a reliable method to determine whether a certain gene variation is present or not. The results do not contain any uncertainties, barring any errors made in the genotyping. If gene variations are determined, then the uncertainty is not caused by the genotyping, but the process that precedes the genotyping: namely the selection of the gene variations that will be determined. If the gene variations that result in altered activity have not all been selected, then an allele in which the selected gene variations do not occur can still have altered activity, namely as a result of a gene variation that was not selected for genotyping. However, the risk of this is low, as the KNMP Pharmacogenetics Working Party always selects the most important gene variations in these cases. If multiple gene variations are determined as is the case for DPYD, then the interpretation of the results can also be a source of uncertainty: it is not certain whether two different gene variations are located on the same allele or not. However, as the dose for patients with two different DPYD gene variations needs to be determined based on the measured DPYD activity, this does not carry any consequences for the genotype-phenotype translation.