Parallel genetic testing for primary lactose intolerance and hereditary fructose intolerance

by Dr Jacqueline Gosink

Gastrointestinal complaints are very common and can be difficult to diagnose. Among the many causes are genetic deficiencies in digestive enzymes. Molecular genetic analysis of polymorphisms in the patient's DNA can determine if inborn enzyme deficits are behind the digestive problems, aiding differential diagnostics. Primary lactose intolerance, for example, is associated with polymorphisms in the regulatory region of the lactase gene (*LCT*), whereas hereditary fructose intolerance (HFI) is caused by mutations in the aldolase B gene (*ALDOB*). A PCR-based DNA microarray provides parallel determination of the two main lactose intolerance-associated polymorphisms (*LCT*-13910$_{C/T}$ and *LCT*-22018$_{G/A}$) as well as the four HFI-associated mutations (A149P, A174D, N334K and del4E4). The fast and simple determination includes fully automated data evaluation, ensuring highly standardized results.

Lactose intolerance

Primary lactose intolerance is a genetically caused deficiency of lactase, the enzyme responsible for splitting lactose into its constituent sugars glucose and galactose. In affected patients, undigested lactose is fermented in the ileum and large intestine, producing by-products such as short-chain fatty acids, methane and hydrogen, which cause the typical symptoms of abdominal pain, nausea, meteorism and diarrhea. Secondary manifestations include deficiencies, for example of vitamins, and as a result unspecific symptoms such as fatigue, chronic tiredness and depression.

Lactose intolerance represents the natural state in mammals. Lactase activity decreases after weaning and in adulthood is often only a fraction of the activity in infancy. Some humans, however, retain the ability to metabolize lactose into adulthood due to specific genetic variants. The frequency of lactase persistence is around 35% worldwide, although it varies greatly between different population groups. It is prevalent in regions with a long tradition of pastoralism and dairy farming, for example in Europe and in populations of European descent. In large parts of eastern Asia, on the other hand, almost 100% of the population is lactose intolerant.

In addition to the primary genetically caused form of lactose intolerance there is also the secondary acquired form. This develops as a result of damage to the intestine, for example from other gastrointestinal diseases such as Crohn's disease, coeliac disease, infectious enteritis or injury from abdominal surgery. The two forms need to be distinguished diagnostically because of the need for different treatment regimes. Whereas individuals with primary lactose intolerance must adhere to a lactose-free or low-lactose diet for life or alternatively take lactase supplements, those with secondary lactose intolerance need only restrict their dairy intake until the intestinal epithelium has regenerated through treatment of the underlying cause.

Diagnostics of lactose intolerance

Classic diagnostic tests for lactose intolerance are the hydrogen breath test and blood glucose tests, with which the patient's ability to metabolize lactose is examined. However, these tests have a low specificity and sensitivity and are influenced by individual factors such as the composition of intestinal flora, colonic pH, gastrointestinal motility and sensitivity to lactose fermentation products. Moreover, they cannot distinguish between the primary and secondary forms

of lactose intolerance. Molecular genetic testing complements these methods, enabling verification or exclusion of primary lactose intolerance with high probability, as well as differentiation of the primary and secondary forms. Genetic testing is, moreover, a non-invasive and more comfortable examination, which does not carry the risk of provoking symptoms of lactose intolerance in non-lactase-persistent individuals.

LCT polymorphisms

The main mutations associated with lactase persistence are *LCT*-13910$_{C>T}$ and *LCT*-22018$_{G>A}$, which are located in the regulatory region of the lactase gene. According to current knowledge, homozygous carriers of the wild-type variants *LCT*-13910$_{CC}$ and *LCT*-22018$_{GG}$ develop lactose *intolerance*, while heterozygous carriers of the variants *LCT*-13910$_{CT}$ and *LCT*-22018$_{GA}$ only show corresponding symptoms in stress situations or with intestinal infections. Homozygous carriers of the mutant variants *LCT*-13910$_{TT}$ and *LCT*-22018$_{AA}$ are lactose *tolerant* as adults. These two polymorphisms are strongly coupled.

Hereditary fructose intolerance

HFI is caused by mutations in the gene for aldolase B, an enzyme essential for fructose metabolism. The mutations result in a reduction or loss in activity or stability of aldolase B, which is responsible for catalysing the breakdown of fructose-1-phosphate (F-1-P) to dihydroxyacetone phosphate and glyceraldehyde. The toxic intermediate F-1-P then accumulates in the body, causing symptoms such as nausea, vomiting and digestive disorders and in the longer term liver damage. HFI is a rare disease, occurring, for example, with a prevalence of 1 in 20000 in Europe. It manifests already in childhood, but may remain undiagnosed due to patients' natural dislike of sweets, fruits and vegetables.

In addition to HFI, intolerance to fructose can also be caused by deficits in the transport of fructose into the enterocytes. This form is known as intestinal fructose intolerance or fructose malabsorption. It is much more common than HFI, occurring with a prevalence of about 30%. It is important to distinguish HFI from fructose malabsorption, because of the resulting difference in dietary requirements. Patients with HFI must completely eliminate fructose and its precursors (e.g. sucrose, sorbitol) from their diet to prevent damage to their organs. Patients with fructose malabsorption, however, should follow a fructose-restricted diet.

Diagnostics of HFI

Intolerance to fructose is usually diagnosed by means of the hydrogen breath test, in which a defined amount of fructose is ingested and then the amount of hydrogen in the exhaled air is measured. In patients with HFI, however, the intake of fructose carries the risk of a severe hypoglycaemic reaction. Therefore, a molecular genetic test for HFI should always be performed before a fructose load test. Early diagnosis of HFI is particularly important to avoid permanent damage to the liver, kidney and small intestine.

ALDOB mutations

In Europe the most frequent mutants associated with HFI are the amino acid substitutions A149P, A174D, N334K (in Human Gene Mutation Database nomenclature) and the deletion del4E4 in the aldolase B gene. For HFI to manifest, both alleles of an individual's DNA must be affected by a mutation. In homozygous genotypes, the two alleles contain the same mutation (paternal and maternal inheritance). If the two alleles exhibit different mutations, this is referred to as a compound heterozygous HFI genotype.

Parallel genetic analysis

Molecular genetic determination of the polymorphisms associated with lactose intolerance and HFI enable diagnosis of these genetic conditions with high certainty. The EUROArray Lactose/Fructose Intolerance Direct enables simultaneous detection of the lactose-intolerance-associated polymorphisms -13910$_{C/T}$ and -22018$_{G/A}$ and the HFI-associated mutations A149P, A174D, N334K and del4E4. Thus, the two genetically caused metabolic disorders can be assessed with a single test.

The test can be performed on whole blood samples, eliminating the need for costly and time-consuming DNA isolation. In the test procedure (Fig. 1), the sections of DNA containing the alleles are first amplified by multiplex PCR using highly specific primers. During this process the PCR products are labelled with a fluorescent dye. The PCR mixture is then incubated with a microarray slide containing immobilized DNA probes. The PCR products hybridize with their complementary probes and are subsequently detected via the emission of fluorescence signals. The data is evaluated fully automatically using EUROArrayScan software (Fig. 2), and in the case of positive results, homozygous and heterozygous states are differentiated. Numerous integrated controls ensure high reliability of results, for example, by verifying that there are no other rare mutations in direct proximity to the tested positions which could interfere with the analysis.

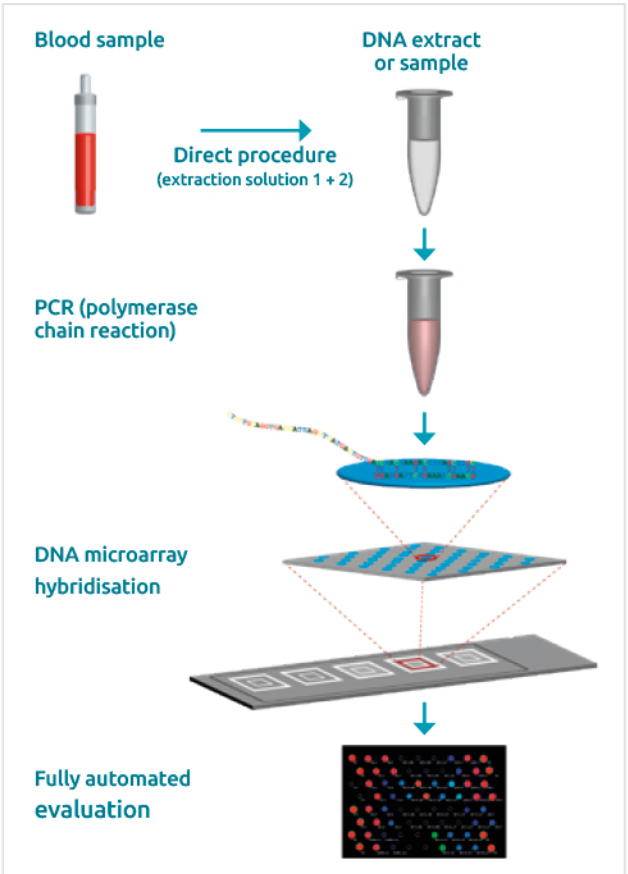

Figure 1. EUROArray procedure

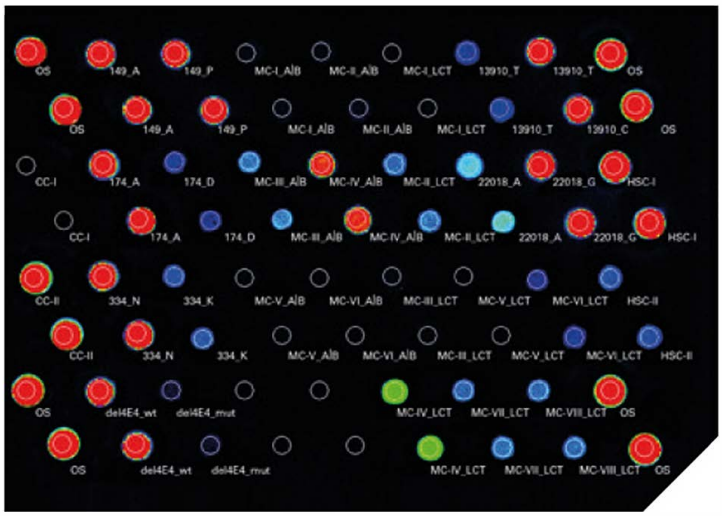

Figure 2. Evaluation of EUROArray Lactose/Fructose Intolerance Direct Dots indicate fluorescence signals at the different probes, including controls, as false-colour representations.

Studies on blood donors

The performance of the EUROArray was investigated using 116 pre-characterized samples from blood donors in Germany and from quality assessment schemes. The EUROArray revealed a sensitivity of 100% and a specificity of 100% with respect to the reference molecular genetic method.

Conclusions

Diagnosis of gastrointestinal disorders often involves a long and challenging process of diagnostic tests and restrictive diets. Since lactose and fructose are widely consumed in many diets, it is important to consider intolerance to these sugars during the diagnostic work-up. Simple genetic analysis enables primary lactose intolerance and HFI to be confirmed or excluded as the cause of gut problems. The parallel analysis offered by the EUROArray enables especially fast and effective diagnostics. Patients diagnosed with these genetic conditions can promptly adapt their diets to ease their symptoms. If the analysis is negative, the physician can focus on searching for other causes of the digestive complaints. The molecular genetic analysis thus provides valuable support for the gastroenterology clinic.

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