wild-type and mutated allele. The Gaucher Disease StripAssay was used to screen a cohort of 91 English Gaucher Disease patients previously genotyped by RFLP. Data obtained with both assays will be presented and discussed with respect to specificity, sensitivity, design and throughput.

P0624. Oculocutaneous albinism in Germany: Spectrum of mutations in the TYR and P gene

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Oculocutaneous albinism (OCA) is an autosomal recessive disorder with heterogeneous genetic background. After extended clinical investigations of 176 unrelated patients [BKK; standardised examination protocol] with albinism we extracted DNA from blood samples for molecular genetic analyses of the genes responsible for OCA1 and OCA2. OCA1 is caused by mutations in the tyrosinase gene (TYR) encoding the key enzyme in melanin biosynthesis. We investigated the 5 exons of the TYR gene by PCR and SSCP and found variations in 66 individuals (1 conformational polymorphism in 46 patients, 2 in 17 patients, and 3 in 3 patients). OCA2 resulting from mutations in the human homologue of the mouse pink eye gene is the most common type of albinism world-wide. Analysing exons 3 to 20 of the P gene (consisting of 25 exons) by PCR and SSCP aberrant signals could be detected in 22 persons (preliminary results: for 21 patients 1 difference to the normal allele was found, one patient showed abnormalities in 2 exons). In 8 patients conformational deviations were detected by SSCP in both the TYR and the P gene. In our sample of German origin about 37% of the patients show SSCP changes in the TYR gene and 12.5% in the P gene (exons 3-20) suggesting that mutations at this locus are possibly underrepresented in Caucasians.
Here, we present SSCP data of the complete TYR and P gene. Differentiation between polymorphisms and relevant mutations was carried out using sequence analysis.

P0625. An Automated Screening Test for Multiple Mutations Associated with Hereditary Iron Overload

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Inherited iron overload is a heterogenous disorder, including “classic” autosomal recessive hereditary haemochromatosis (HH), as well as juvenile and autosomal dominant forms of the disease. The most prevalent variant among Caucasians is autosomal recessive HH due to mutations in the HFE and transferrin receptor 2 (TFR2) genes. More recently, mutations in the genes for ferroportin (FPN1/SLC11A3/IREG1) and ferritin heavy chain (FTH1) were found to be associated with autosomal dominant iron overload. In most cases therapeutic phlebotomy provides an effective and inexpensive lifelong treatment. DNA testing is now routinely used to support the diagnosis in patients with abnormal iron parameters, for the presymptomatic identification of individuals at risk, and its potential for population screening programs is currently under discussion.
We have developed a reverse-hybridization assay (Haemochromatosis StripAssay) for the rapid and simultaneous detection of 18 known mutations in the HFE, TFR2, FPN1 and FTH1 genes. The test is based on multiplex DNA amplification and ready-to-use test strips containing oligonucleotide probes for each wild-type and mutated allele immobilized as parallel lines. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot).
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P0626. Mutation Screening For Tyrosinaemia Type I

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Tyrosinaemia Type I is caused by a deficiency of the enzyme fumarylacetoacetase (FAA), the last enzyme in the catabolic pathway of tyrosine. As with most other inborn errors of metabolism, carrier status cannot be reliably excluded using biochemical markers (ie metabolites or enzymes), and is a particular issue for consanguineous families. Prenatal diagnosis by biochemical methods may also be problematic for some families.
The FAA gene maps to 15q23-25, has 14 exons, and more than 34 different mutations have been reported. There are four common mutations associated with this disorder, most of the remainder are private mutations. Of the 37 patients (from 35 families) at Birmingham Children’s Hospital, 62% of disease alleles were accounted for by these four mutations. 27% were G192T, 27% IVS12+5 G to A, 6.8% IVS6-1 G to T, and 1.4% G1009A. 68% of these patients are of Asian origin: within this group G192T is exclusive, and IVS12+5 G to A is more common than in the whole group.
Mutation screening by single-stranded conformational polymorphism (SSCP) analysis was developed to identify the remaining disease-causing mutations. 8 patients had further testing (14 untyped alleles). From this group of patients, 9 additional mutations were identified of which 6 were novel, and together accounted for 13 of the 14 disease-causing alleles. Five of the mutations were mis-sense mutations (S23P, H133R, P156Q, T325M and S352R) and one splice site mutation (IVS9-2 A to G). These findings have led to an improved approach to diagnosis of tyrosinaemia type I for clinical practice.

P0627. Tetrahydrobiopterin Deficiencies in the Maltese Population

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A higher than usual frequency of hyperphenylalaninaemia due to tetrahydrobiopterin (BH_4) deficiencies, specifically Dihydropteridine Reductase (DHPR) deficiency, is present in the Maltese population. Classical Phenylketonuria due to Phenylalanine Hydroxylase deficiency has not been identified to date.
Molecular analysis of the DHPR gene in 3 families (4 probands born to unrelated parents over a span of 4 years) has identified the G23D mutation, a previously identified mutation in 2 Italian and 1 other Maltese patient. This glycine to aspartic acid change at the 23rd amino acid in the protein alters a highly conserved amino acid in the NADH binding domain of the DHPR gene.
Population studies have shown this mutation to be abnormally frequent in the Maltese population. A heterozygote carrier rate of 2% has been established in a cohort of 400 random Maltese neonatal DNA samples. This mutation is of Mediterranean origin and is a clear example of a founder effect.
A neutral polymorphism, L132L, in the DHPR gene had previously been identified in a patient carrying the G23D mutation. This polymorphism was not present in our DHPR patients, however 6 out of 7 patients manifesting clinical symptoms typical of Dopa Responsive Dystonia (DRD) also had the L132L polymorphism in either homozygosity or heterozygosity. DRD in these 7 patients, from 4 unrelated families, is believed to be due to GTP Cyclohydrolase I deficiency - the first enzyme in the biosynthesis pathway of BH_4. Molecular and biochemical analysis are currently being carried out to identify the causative mutation in these patients.

P0628. Detection of two novel large mutations in SLC7A9 by semi-quantitative fluorescent multiplex PCR

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Cystinuria is an autosomal recessive aminoaciduria in which two clinical types have been described: I and non-I. Mutations in the cystine and dibasic amino acid transporter cause cystinuria: mutations in the heavy subunit, rBAT, coded by SLC3A1 gene, cause type I cystinuria while mutations in the light subunit bo,+AT, coded by SLC7A9 gene, cause non-type I cystinuria. Using multiplex semi-quantitative fluorescent PCR we have amplified the 13 exons of SLC7A9 together with exon 5 of DSCR1 (located on chromosome 21) as a double dose control gene. The PCR products were loaded in a 48-well acrylamide gel together with an external fluorescent size standard and run in an ABI PRISM 377 DNA sequencer. The results were processed by GENESCAN™ software. With this technique we have detected two novel large mutations in 2 Spanish families: a 5kb deletion and a 5kb duplication, both affecting exon 12, originated