WS11.1 Development of a comprehensive workflow for the analysis of CFTR gene using next generation sequencing technology

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Objectives: Molecular diagnosis of cystic fibrosis (CF) is essential for the provision of genetic counselling, prenatal diagnosis, cascade screening in families, as well as for understanding the genotype-phenotype relationship. The large number of different mutations in CFTR gene makes the genetic diagnosis of CF difficult. From the start of molecular diagnostics CF, many different techniques (ARMS, reverse hybridisation, SSCP, DGGE, HRM, MLPA, direct sequencing) have been applied to detect CF mutation. Next-generation sequencing (NGS) technology enables us to improve efficiency of current methods.
Methods: We evaluated in our laboratory the multiplex PCR strategy (CFTR MASTER, Multiplicon) to generate the patient's library followed by pyrosequencing using a NGS platform (454 GS Junior, Roche) and subsequent bioinformatics analysis based on the software Sequence Pilot (JSI Medical Systems). All sequence changes were confirmed by Sanger sequencing.
Results: We have assessed the suitability of this workflow to be applied in CF molecular diagnostics using a cohort 24 CF patients displaying a severe respiratory and digestive phenotype. The NGS sequencing data provided an adequate depth of sequencing coverage to accurately detect the CFTR exome. Common and rare CF mutations and coding SNPs were detected. Three novel sequence changes we identified, two missense c.729G>A, c. 2834C>T, and c.2537–2547delinsTCGGTCACAAGAG mutations. This sequence changes have been tested by conservation study to elucidate their significance as a CF causative mutations.
Conclusion: NGS is changing genetic diagnosis CF due to its huge sequencing capacity, cost-effectiveness, precise and quick results.

WS11.2 Age related cut-off levels for immunoreactive trypsin (IRT) in healthy newborns in the first two months of life

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Background: Newborn screening (NBS) for CF, based on an IRT-DNA-IRT protocol, was introduced in Switzerland in 2011. In the DNA panel used in our NBS, only the seven most common Swiss CFTR mutations were analyzed. If IRT was above the cut-off (>50 ng/ml) and no mutation was detected, a second IRT filterpaper specimen was requested. So far there are no data on IRT levels in newborn babies aged several weeks. We aimed therefore at calculating the percentiles for IRT in the first two months of life in relation to the age at sampling.
Methods: The second IRT specimens were usually collected by the family physician in the 3rd to 5th week of life and mailed to the NBS laboratory. IRT was measured with the Neonatal IRT Kit on the GSP Instrument both from PerkinElmer (Turku, Finland). Analysis was done on the day the specimens arrived in the laboratory. Babies with an IRT >50 ng/ml were considered screening positive and referred to the CF center for sweat test (ST). The screening negative results were divided in four groups according to the age at sampling: 15–21 d, 22–28 d, 29–35 d and >36 d.
Results: By December 2012, 511 IRT results were collected and assigned to one of the four groups as follows: 214, 180, 85, and 32. Mean IRT values of the groups were 25.6, 22.2, 19.9 and 17.9 ng/ml, respectively. The 99.9 Percentile for each group was 49.8, 49.0, 37.2, and 29.6 ng/ml.
Conclusions: Our results document the significant decrease of IRT levels in healthy babies during the first two months of life. When performing NBS for CF using repeated IRT measurements, the decline of IRT in relation to the age at time of sampling has to be taken into account when interpreting the results.

WS11.3 Change of algorithm in the CF centers influences the amount of equivocal CF diagnoses in the newborn screening program in Switzerland

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Background: Newborn screening (NBS) for CF, based on an IRT-DNA-IRT protocol, was introduced in Switzerland in 2011. The goal of our program is to detect all children with CF but not equivocal CF (ECF). It was closely monitored and evaluated after 12, 24, and 36 months. Based on the evaluation, we adapted the program after 1 and 2 years. The aim of this study was to investigate the ratio of CF to ECF diagnoses in the 3 time periods.
Methods: All children with a positive screening result were referred to a CF center for a sweat test (ST). In the first year (2011), an extended DNA analysis was performed when the ST was positive or borderline. This led to long delays when STs had to be repeated. In 2012, we performed extended DNA analysis immediately when STs were inconclusive or impossible, to reduce the waiting time for a final diagnosis. A consecutive increased number of ECF in 2012 led to a further change in algorithm: When the ST was not possible, we determined fecal elastase (FE) before extensive DNA analysis. When FE was negative we repeated the ST when the child's weight was >4000 g (year 2013).
Results: By December 2013, 256 children had been screened positive and referred to a CF center for investigations. In 73 CF was confirmed, 10 had an ECF and 160 children were negative (1 child moved abroad, 1 died, 11 not yet fully investigated). In 2011, the ratio CF/ECF was 8:1 (27/3), in 2012, 4:1 (26/6), and in 2013 20:1 (20/1).
Conclusion: In addition to the screening process in the laboratory, the algorithm used in the CF centers can strongly affect the ratio of CF versus ECF. With a close monitoring and simple adaptions, the number of ECF can be reduced.

WS11.4 Uptake of cascade testing in CF families: preliminary results from the experience of western Brittany, France

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Objectives: Diagnosis of CF allows relatives of the patient to know their status regarding the mutation that segregate in their family. This test (called family or cascade carrier testing) enables detection of new 1-in-4 risk couples and thus prevention in families. Our study aimed to report the uptake of carrier testing in an area where CF is frequent (Finistère, western Brittany, France).
Methods: All patients born in our district between 1980 and 2004 were eligible for the present study. Here we presented data from the first 12 families included. Family trees were faced with the carrier tests made in those families in the sole genetic laboratory of our area.
Results: To date, 320 family members have been identified, and 145 were eligible for testing (adult, living in our district, alive at time of diagnosis). Of them 72 performed the test, leading to a percentage of uptake of 49.7% (95% CI: [41.5%; 57.8%]). Testing identified 39 carriers (54.2%). Among them 37 were in couples and 27 had their spouse screened. Finally, we identified 2 1-in-4 risk couples (1 sister and 1 uncle/aunt couples), who requested prenatal diagnosis 6 times. Four affected pregnancies were detected and terminated. Moreover, family testing enables 25 heterozygote couples and 101 negative relatives (by testing or by deduction) to be ascertained (total information rate 43.8%).
Conclusion: We assess, for the first time in Europe, uptake of family testing and its public health implications. These preliminary results tend to reveal a higher uptake in our area than previously reported in Australia (Victoria State: 11.8%).
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