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建立快速分子診斷 JAK2 V617F 突變檢驗方法

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Establishment of the rapid molecular detection of JAK2 V617F mutation

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BACKGROUND: Chronic myeloproliferative disease (CMPD) is a group of malignant blood disorders including polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic myeloid leukemia. CMPD is characterized by proliferation of one or several lineages in hematopoietic system. The pathogenesis of CMPD is not clear, recent years, more studies demonstrated that CMPD have a higher mutation rate of gene JAK2 V617F. METHODS and MATERIALS: We evaluated JAK2 V617F mutation molecular diagnosis method. Twenty-nine patients diagnosed with CMPD were examined. The research project was reviewed and approved by the IRB committee of our hospital (DMR99-IRB-108). We have developed an allele-specific-PCR assay. All mutations are confirmed with directly sequencing. RESULTS: Herein, we established a rapid detection molecular diagnosis about JAK2 V617F mutation assays for CMPD. Analysis of a series of 29 patient specimens revealed 11 positive for JAK2 V617F mutation and 18 negatives. CONCLUSIONS: This study demonstrates that the allele-specific-PCR assay could detect mutations of JAK2 V617F in CMPD patients. We expect to set up the molecular diagnostic technique for clinical laboratory service.

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建立台灣女性乳癌易感基因座 2q35, 3p24, 17q23 和 FGFR2 基因檢驗

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Replication of Susceptibility loci on 2q35, 3p24, 17q23 and FGFR2 gene in Taiwanese breast cancer females

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BACKGROUND: The breast cancer is the public health subject which the global woman and the government cared. In recent years, the genome-wide association studies (GWASs) development provided lot of advantageous evidences. In recent years, mounting evidence identified that 2q35, 3p24, 17q23 and FGFR2 may be genetic susceptibility loci for breast cancer. The aim of this study is to evaluate the association of four polymorphic genotypes in these loci, with breast cancer among Taiwanese female. METHODS and MATERIALS: We evaluated these breast cancer risk genes, including 2q35-rs13387042, 3p24 -rs4973768, 17q23-rs650490 and FGFR2-rs2981578. Eighty-nine patients diagnosed with Breast cancer (Mean age 53 years old) were examined. The 2q35, 17q23 and FGFR2 were detected by using PCR-RFLP and 3p24 was detected by ACRS methods. All mutations are confirmed with directly sequencing. RESULTS: Herein, we established of the molecular diagnosis about four breast cancer risk gene (rs13387042, rs4973768, rs650490 and rs2981578). The allelic frequency analysis showed that allele A of 2q35 conferred an increased odds ratio of 2.95-fold for breast cancer risk than allele G (OR=2.95, 95% CI=1.29- 6.71, P=0.008), while those for 3p24, 17q23, and FGFR2 were not significant (P>0.05). The 2q35 -rs13387042 was susceptible associated with breast cancer. CONCLUSIONS: This study demonstrates that the 2q35 allele A may be a potential biomarker for breast cancer risk and its role in breast carcinogenesis needed further confirmation.

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建立新穎分子診斷檢測急性淋巴性白血病 NPM1 突變檢驗方法

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Establishment of a novel molecular diagnosis method to detect NPM1 mutation in acute myeloid leukemia

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BACKGROUND: Molecular characterization of acute myeloid leukemia allows prognosis stratification and potentially can alter treatment choices and pathways. Mutations in exon 12 of the nucleophosmin gene (NPM1) that cause the encoded protein to abnormally relocate to the cytoplasm are found at diagnosis in about 50% of karyotypically normal acute myeloid leukemias and are associated with a more favorable outcome. METHODS and MATERIALS: We evaluated NPM1 mutation molecular diagnosis method. Twenty-one patients diagnosed with AML were examined. The research project was reviewed and approved by the IRB committee of our hospital (DMR99-IRB-108). We have developed a PCR-based assay for NPM1 exon 12 mutations and detected by using PCR-Sequence methods. RESULTS: Herein, we established molecular diagnosis of NPM1 mutation assays for AML. Analysis of a series of 21 patient specimens revealed 6 positive for NPM1 mutation and 21 negatives. In additional, NPM1 positive mutation is referred to as TCTG (Type I) and GTCG heterozygote insertion were detected from 5 and 1 patients, respectively.
CONCLUSIONS: This study demonstrates that the PCR-based and sequencing methods could detect mutations of NPM1 exon 12 in AML patients. We expect to set up the molecular diagnostic technique for clinical laboratory service.

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從自發性矮小及生長激素缺乏的病人中探討其 leptin 的濃度與基因多型性

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Study of the leptin levels and its gene polymorphisms in patients with Idiopathic Short Stature and Growth Hormone Deficiency

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A significant correlation between leptin levels and growth hormone (GH) secretion has been documented. We hypothesize that the phenotype of GH deficiency (GHD) and idiopathic short stature (ISS) may be associated with polymorphism in the leptin gene. The prevalence of a single nucleotide polymorphism (SNP) in the leptin gene (LEP) promoter at -2548 and the leptin concentrations in GHD and ISS were compared to those of healthy controls. IGF-1 and leptin concentrations were significantly lower in both the GHD and ISS groups than in the control group. The ISS and GHD groups had a significantly different distribution of SNP alleles at the LEP -2548 (P=0.010). Individuals with LEP-2548A/G or G/G genotype in ISS group (47.5%) showed a significantly lower weight and BMI (but not leptin levels) than individuals carrying the A/A genotype (52.5%). LEP-2548A/A in GHD patients (65.8%) was associated with lower weight and BMI and associated with lower leptin concentrations than individuals carrying A/G or G/G genotype (34.2%). These data suggest that the LEP -2548A polymorphism may associate with thegrowth of the chilfren wih ISS and GHD.

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中台灣地區地中海型貧血之產前基因診斷

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Prenatal Diagnosis of Thalassemia in Mid-Taiwan

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Introduction：In Taiwan, the carrier percentage of α0-thalassemia, α+-thalassemia and β-thalassemia are 4%, 1%, and 1.5%, respectively. α0 -thalassemia carriers, around 95% is SEA type, 4% is Philippine type, and the other is Thailand type. However, in α+-thalassemia genotype, single α gene deficiency is the major type, such as Hb Quong-Sze or Hb Constant-Spring. The other β-thalassemia, there are more than 20 types have been discovered in Taiwan. There are 4 common types, IVS-2 nt654 C>T, codon 41/42-TCTT, promoter-28 A>G, codon 17 A>T, have occupied 92%. Methods：From 1999 to 2010, 1125 prenatal cases were collected from several cohorts of patients or subjects. α-thalassemia genotype by Gap-PCR-based methods, β-thalassemia genotype by ACRS or PCR-RFLP to discover the location for β-thalassemias and DNA sequencing analysis is used to confirmed β-thalassemias major. Result：Over the past 10 years, including amniotic fluid, chorionic villus sampling (CVS), and cord blood samples. α genotype has 385 cases of SEA type, 191 cases of Hydrops, 19 case of α-Philippine type, 5 case of Thailand type. In additional, β genotype including 19 cases of Promoter -28 minor, 42 cases of IVS-2 nt654 minor, 34 cases of codon 41/42 minor, 11 cases of codon 17 minor. α combined with β genotype frequency includes 1 case of SEA combined with β-start codon, 4 cases of SEA combined with IVS-2 nt654, 3 cases of SEA combined with codon 41/42, 4 cases of SEA combined with Promoter -28, 3 cases of SEA combined with HbE and 2 cases of α3.7 deletion combined with IVS-2 nt654 . Conclusions：Herein, prenatal diagnosis of the α-thalassemia major with frequencies of Hydrops was the largest, 191 cases; α-thalassemia carrier, 385 cases of SEA type was the most. In additional, β-thalassemia major with frequencies of 11 cases of IVS-2 nt654 combined with codon 41/42 major was the most, and the second was the IVS-2 nt654combined with P-28 major, 10 cases.

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鑲嵌型環狀第 13 號染色體合併小頭症患者之極小致病區域之探討

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Smallest Critical Region for Microencephaly in a Patient with Mosaic Ring Chromosome 13, r(13) Duplication/Deletion and Disomy 13

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A ring chromosome 13 or r(13) exhibits breakage and reunion at the breakpoints on the long and short arms of chromosome 13, with deletions of the chromosomal segments distal to the break points. Ring 13 chromosome accounts for about 20% of ring chromosomes that are compatible with life. We report on a female patient with mental retardation, growth retardation, microencephaly, craniofacial dysmorphy, hearing impairment, and prolonged prothrombin time. The peripheral blood lymphocytes revealed a karyotype of 46,XX,r(13)(p13q34)[71]/45,XX,-13[12]/ 46,XX,dic r(13;13)(p13q34;p13q34)[9]/46,XX,-13,+ mar [5] /47 ,XX, + r(13)(p13q34)×2[2]/46,XX[1] at age 6yrs and 46,XX,r(13)(p13q34)[82]/45,XX,-13[14]/46,XX,dic r(13;13)(p13q34; p13q34)[2]/46,XX,-13,+mar[2] at the age of 14. Array comparative genomic hybridization analysis of the blood demonstrated a 4.37 Mb deletion at chromosome 13q [arr cgh 13q34q34(109,743,729-144,110,721)]. This case combined with 14 previous cases indicates that the smallest critical region for chr13 microencephaly was 109,743,729-144,110,721.

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平輕型巴金森氏病的分子診斷

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Molecular Diagnosis of Young Onset Parkinson's Disease

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Background: Parkinson' s disease (PD) is the second most frequent neurodegenerative disorder after Alzheimer' s disease. Although PD appears to be sporadic in most cases, several causative genes and susceptibility factors have been identified that cause familial forms of the disease with Mendelian inheritance with autosomal dominant or autosomal recessive inheritance. Approximately 5~10% of patients with the clinical picture of PD carry a mutation in one of the known genes that cause monogenic forms of the disorder. Onset of primary parkinsonism before 50 years is known as early onset parkinsonism (EOP). Methods: Our study is aimed to screen the clinical diagnosed familial EOP for the common mutation sites by PCR/DNA sequencing. The genes for screening are Parkin, PINK1, and ATP13A2. Results: In this study, screening twenty-five patients with clinical diagnosis of Parkinson's disease (also has a family history and early onset). Two patients were not fount any mutation site at out study (diagnosis sensitivity: 92.0%). Totally eight mutation patterns were found: Parkin S167N heterozygote (seven patients), Parkin S167N heterozygote and PINK1 A340T heterozygote(six patients), PINK1 A340T heterozygous (three patients), Parkin S167N homozygote (three patients), The following four kind of variants only have one patient: Parkin V380L heterozygous, Parkin S167N heterozygote and PINK1 A340T homozygous, Parkin V380L heterozygote and PINK1 V350L heterozygote, Parkin S167N heterozygote + Parkin V380L heterozygote+ PINK1 A340T heterozygote. No mutation in ATP13A2 was found Conclusion: At least, 13 loci and 9 genes in PD are reported. This method only examines seven mutation sites of three genes, was unable to cover all PD genes. However, the diagnostic sensitivity is 92%. The method is required to detect gene mutation and clinical relevance, not to use for disease diagnosis. The gene mutation sites could be used for support of clinical diagnosis.

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比較不同分子病理學技術進行石蠟包埋檢體之滑液肉瘤檢測成效

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Efficacy Comparison of Different Molecular Pathology Techniques on Detection of Synovial Sarcoma in Formalin-Fixed Paraffin-Embedded Sample

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滑液肉瘤是惡性程度較高的軟組織惡性腫瘤，源於關節及滑膜組織，好發於年青人，其中最常見的單相纖維型(MFSS)與惡性外周神經鞘膜瘤、纖維肉瘤等梭形細胞間葉腫瘤在形態學上極爲相似，因而診斷較爲困難；近年來研究指出，90%以上的滑液肉瘤常伴隨染色體易位 t（x;18）（p11;q11）的發生，並且衍生出 SS18-SSX1、SS18-SSX2 及少數的 SS18-SSX4 融合基因；此外，滑液肉瘤中也被偵測到有大量的 TLE1 蛋白質表現。因此，有效的檢測出染色體異位、SS18-SSX 融合基因表現及 TLE1 蛋白質表現，能夠幫助滑液肉瘤在臨床上的診斷。 目前用於滑液肉瘤的診斷方法有免疫組織化學染色(IHC)及分子病理檢測，如螢光原位雜交(FISH)、反轉錄聚合酶連鎖反應(RT-PCR)以及即時反轉錄聚合酶連鎖反應(RRT-PCR)等技術。因此本研究分別使用上述方法分析林口長庚醫院 64 位組織病理學診斷爲滑液肉瘤的病人中 TLE1 蛋白質表現、染色體易位及 SS18-SSX 融合基因表現，並進一步比較不同檢測方法的檢出率。 以臨床診斷爲依據，分析各病理檢測法之敏感度及特異性；敏感度分析結果顯示如下，TLE1 IHC 爲 85.94% (55/64)，FISH 爲 59.37% (38/64)，傳統 RT-PCR 爲 68.75%(44/64)，RRT-PCR 爲 73.43% (47/64)。專一性分析結果顯示各項分子病理檢測法專一性皆達 100% (6/6)；而 TLE1 IHC 檢測法專一性較差，且有 18.8% (12/64)呈僞陽性反應。 總結本研究結果，雖然 TLE1 IHC 結果專一性較差，但利用 TLE1 IHC 進行篩檢，配合 RRT-PCR，能夠有效增加滑液肉瘤檢測之敏感性達到 93.75% (60/64)。結合組織病理學診斷（TLE1 IHC）之高敏感度及分子病理學技術（RRT-PCR）之高特異性可提供臨床滑液肉瘤檢測診斷及治療的重要參考依據。

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利用 Real-Time RT-PCR 檢測人類冠狀病毒 NL63

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Detecting of Human Coronaviruses NL63 by Real-Time RT-PCR

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Human coronavirus NL63 (HCoV-NL63) is one of common human respiratory pathogens, causing upper respiratory tract infection, bronchitis, and pneumonia. HCoV-NL63 spreads around the world, but no antiviral drugs or vaccines are available to treatment now. In order to investigate the epidemiology and prevalence of human coronavirus NL63 in Taiwan, we examine the prevalence of HCoV-NL63 in nasopharyngeal specimens using real-time RT-PCR. Two pairs of primers based on the nucleocapsid gene were designed for real-time RT-PCR. Agarose gel electrophoresis demonstrated RT-PCR products as the size of 120 bp and 251 bp, respectively. The melting peaks indicated Tm values of real-time RT-PCR products at 78℃ and 79.5 ℃, respectively. The primer pairs using real-time RT-PCR showed high sensitivity (at least 10 pfu) and specificity with no cross-reactivity of Japanese encephalitis virus, Influenza virus, Coxsackie virus 16, and Enterovirus 71. In addition, 83 nasopharyngeal/throat specimens collected from inpatients and outpatients in CMUH were no-detectable for HCoV-NL63. We still examine the prevalence of HCoV-NL63 in central Taiwan.

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以第二代與第一代 COBAS AmpliPrep/COBAS TaqMan HIV-1 試劑組檢測血漿中 HIV 病毒量之比較

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Comparison of COBAS AmpliPrep/COBAS TaqMan HIV-1 v2.0 and v1.0 for detecting HIV viral loads from plasma specimens

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Introduction: Quantitative measurements of HIV viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV disease, and that reductions in plasma virus levels may be associated with decreased risk of clinical progression. COBAS AmpliPrep/COBAS TaqMan HIV-1 v2.0 (CAP/CTM HIV v2.0) is a new commercial kit for determination of the viral load (VL) in plasma are able to detect 20 copies of HIV-1 RNA/ml. Methods: In this study, 202 clinical HIV specimens have been collected. Parallel tests of COBAS AmpliPrep / COBAS TaqMan HIV-1 v1.0 (CAP/CTM HIV v1.0) and the CAP/CTM HIV v2.0 experiments have been performed on each sample simultaneously. Results: The results showed the highly correlation between CAP/CTM HIV v1.0 and CAP/CTM HIV v2.0 (R2 = 0.9142). During these correlation experiments, we found 6 specimens in low HIV titers have different results between these two kits. All 6 specimens with CAP/CTM HIV v2.0 are in linearity range, but they are "Target NOT detected" or "less than the instrument detection limit (<40 copies / ml)" in CAP/CTM HIV v1.0. We use two concentrations of self-prepared pool plasma for reproducibility analysis. The reproducibility results of CAP/CTM HIV v2.0 demonstrated both SDs were 0.07 and 0.11, and CVs were 2.03% and 2.29%, respectively. Conclusion: After comparative analysis, the CAP/CTM HIV v2.0 is not only better sensitivity than CAP/CTM HIV v1.0, but also shows a good reproducibility, In conclusion, CAP/CTM HIV v2.0 could replace CAP/CTM HIV v1.0.

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快速分子診斷 CMV 感染角膜葡萄膜炎之經驗

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Experiences for rapid molecular detection of CMV related keratouveitis

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Introduction: Cytomegalovirus (CMV) related corneal endotheliitis and anterior uveitis could be found in immunocompetent patients, although the symptoms of these ocular involvements are comparatively mild and less common. In Oct of 2008, a 44-year-old male with CMV related anterior uveitis, which was diagnosed by a positive result of PCR in aqueous humor. Thereafter, we began receiving the aqueous of patients with suspected viral keratouveitis by ophthalmologist for CMV-PCR examination. Methods: A total of fifty-two patients suspected of CMV keratitis or keratouveitis were collected from Oct 2008 to Jul 2011. The detection analysis of the viral DNA were performed home brew CMV PCR assay, further then quantified assay by the Nanogen CMV Q-PCR kit. Results: Analysis of a series of 52 patient specimens revealed 20 positive for CMV PCR, and positive rate is about 38%. All results were correlated with the clinical data and the disease. In additional, we use CMV Q-PCR to detect viral load of 11 positive specimens. The viral loads demonstrate about 557~23,100,000 copies/mL. Conclusion: The CMV PCR appears as a fast and accurate method for an exact identification of the viral DNA from patient aqueous humor with CMV keratouveitis. The CMV Q-PCR quantification is important for the treatment evaluation and for the specification CMV related keratouveitis and lower the rate of delayed diagnosis.

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首例成功利用分子診斷方式執行胚胎植入前篩檢「急性間歇性紫質症」

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Successful preimplantation genetic diagnosis of acute intermittent porphyria by amplification refractory mutation system-quantitative polymerase chain reaction (ARMS-qPCR)

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Acute intermittent porphyria (AIP; MIM # 176000), an autosomal dominant inborn error of metabolism, is caused by partial deficiency of hydroxymethylbilane synthase (HMBS) affecting heme biosynthesis. The disease has large phenotypic variability among affected individuals, determined in part by environmental, metabolic, and hormonal factors. Clinical onset of the disease always happens during or after puberty and is characterized by intermittent attacks of neurological dysfunction. Mutations of HMBS gene are known to be responsible for the disorder, and the genetic diagnosis of this gene is crucial as the principal manner of medical management is the avoidance of specific precipitating factors. A Taiwanese couple was referred to our center for preimplantation genetic diagnosis (PGD). Preliminary molecular analysis show the wife has a heterozygously nonsense substitution, c.848G>A (p.W283X) in HMBS gene, which has been reported as pathogenic mutation. We then developed an in-house duplex-nested amplification refractory mutation system polymerase chain reaction (ARMS-PCR) combining with real-time quantitative PCR and direct sequencing to detect the disease-causing mutation in embryos acquired after ovarian stimulation. In each embryo, a single blastomere was biopsied at the eight-cell cleavage stage. Of the six blastomeres examined, two were found to be unaffected and the corresponding embryos were selected for implantation at day 5 after informed consent. A successful singleton pregnancy was eventually achieved. After 40 weeks of uneventful gestation, the woman gave birth to a healthy female baby, whose postnatal genotyping confirmed the results of PGD and amniocentesis. To our best knowledge, this is the first successful PGD case reported in AIP. We propose that the molecular technique reported in this study will significantly contribute to prenatal and preimplantation analysis of AIP.