行政院國家科學委員會專題研究計畫 成果報告

台灣智能障礙族群基因體失衡之研究
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 98-2314-B-039-003-
執 行 期 間 ：98 年 08 月 01 日至 99 年 10 月 31 日
執 行 單 位 ：中國醫藥大學基礎醫學研究所

計 畫 主 持 人：林齊強
共 同 主 持 人：蔡輔仁、李月君
計畫參與人員：碩士級-專任助理人員：劉乃甄

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 99 年 11 月 18 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

台灣智能障礙族群基因體失衡之研究

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執行機構及系所：中國醫藥大學 基礎醫學研究所
中山醫學大學
計畫主持人：林齊強 教授(中國醫藥大學 基礎醫學研究所)
共同主持人：蔡輔仁 教授(中國醫藥大學 學士後中醫系)
李月君 教授(中山醫學大學 生物醫學科學學系)
計畫參與人員：劉乃甄

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（一）中文摘要

背景：智障患者是終生遭受智能障礙，其發生率在一般族群中為 2-3%，其中一半以上其智障病因不明，雖然超過 50%先天性智障者若其 IQ 少於 50，其致病原因可能與基因體缺陷有關，然而微基因體異常是無法用傳統細胞遺傳分析方法偵測出來的。最近由於分子細胞遺傳學的進步加上全基因體比較雜交晶片技術之發展，已可以應用來偵測及鑑定智障者之微基因體異常。

目標：本計劃目標是完成約 100 位智障者之全基因體晶片分析。這些智障者是由本土一個智障族群篩選出來。計劃目的是針對偵測率，基因體位置倍數變異(CNC)的大小及其中包涵有功能性或有相關病理性的基因之收集與評估。

方法：應用晶片型全基因體分析法(採用 Affymetrics 和 Agilent 二種平臺) 從事基因體失衡分析，並用 BAC clone 螢光原位雜交法來鑑定所發現之 CNC。

結果：本計畫完成約 95 位智障者之全基因體晶片分析。發現 14 位先天性智障者有基因體失衡(14/95)，偵測率到達 14.7%。總共有 23 個染色體位置倍數出現有擴增(56 Kb-3.46 Mb)或缺失(27.5 Kb-5.03 Mb)。這些染色體位置至少包涵有 53 個有功能性基因，其中有 15 個可能和神經細胞發育有關。

重要性：本研究可能導致對有關智障病理性 CNC 的發現，有助於對神經系統發育相關基因訊息傳達之研究。因此會進一步了解智障致病機制，對智障之診斷、療治及處理上將有所貢獻。

關鍵詞：先天性智障、晶片型全基因體比較雜交法、螢光原位雜交法、病理基因體位置倍數變異、基因體失衡病變。

（二）Abstract

Back ground: Mental retardation (MR) is a life-long mental handicap that occurs in 2-3% of the general population. More than one-half of the MR cases their etiology is unknown. Although over 50% of idiopathic MR with IQ less than 50 is likely to have genomic defects, and yet such defect at submicroscopic level can not be detected by conventional cytogenetic study. Recent advances in molecular cytogenetics analysis and genome-wide array-based comparative genome hybridization (aCGH) technology, submicroscopic genomic disorder bases of MR can be identified and characterized.

Specific Aim: In this proposed study, our goal is to complete the chromosomal microarray (aCGH) analysis on ~100 idiopathic MR patients selected from a cohort of mental retardation patients. We aim at collecting data on the diagnostic yield, CNC size and functional genes or potential candidate genes involved in the CNC region detected.

Method: Chromosomal microarray analysis using to genome-wide microarray platforms (Affymetrics and Agilent) has been conducted. Verifications of the CNV observed were performed by BAC clone FISH study.

Results: The chromosomal microarray profiles of 95 idiopathic MR patients were obtained. Copy number changes (CNC) were detected in 14 MR patients (14/95) with diagnostic yield of 14.7%. A total of 23 chromosomal sites were found either to have duplication or deletion. The sizes of duplication range from 56 Kb to 3.46 Mb and the sizes deletion range from 27.5 Kb to 5.03 Mb. At least 53 functional genes are involved in those CNC sites and 15 of those genes could be potentially associated with neuronal cell development.

Significance: The study will lead to the identification of potential pathogenic CNCs for MR. This in turn could lead to the discovery of a network of neuro-developmentally associated genes that turn helps us to understand the etiology of MR disorders and will assist us in diagnosing, managing and treatment of the disorders.

Key words: mental retardation, chromosomal microarray, BAC clone FISH, copy number change (CNC), genomic disorders.

（三）報告內容

前言 (Introduction):

Mental retardation (MR) is a life long disability which has a enormous affect to the patients, their families and the society. It is neurodevelopment disorder that involves complex cognitive and adaptive impairments. About half of the MR cases, the etiology are unclear. It has been estimated that over half of idiopathic MR with IQ less than 50 may have genetic defects (Flint and Wilkie, 1996). Two largest groups of MR are well known to have a chromosomal (genetic) defect, the Down syndrome and the fragile X syndrome. However, more than 500 other clinical disorders also have been associated with MR (Wahlstrom, 1990; Winter and Baraitser, 1991) and many of idiopathic MR may also etiologically heterogeneous. A number of genetic diseases associated with MR have been shown to arise from dosage imbalance of one or more developmental important gene(s). Such genomic disorders are estimated to occur at a frequency of 0.7 to 1 per 1,000 live births (Lupski 1998; Ji et al., 2000). Some well characterized genomic imbalance disorders associated with MR including Prader-Willi and Angelman syndrome, Williams-Beuren syndrome, Smith-Magenis syndrome, DiGeorge and velocardiofacial syndrome and Cat eye syndrome may have submicroscopic deletion or duplication that can not detected by conventional karyotypic analysis. With the recent advances in molecular cytogenetics study and microarray array comparative genomic hybridization aCGH analysis, a portion of genomic disorder basis of MR has been identified and characterized.

研究目的 (Research Goal)

The **goal** of this research proposal is to perform genome-wide high resolution, oligonucleotide-based array comparative genomic hybridization (aCGH) study on a cohort of idiopathic MR population from Taiwan. We plan to complete the analysis of 195 selected MR patients (numerical and structural aberrations had been rule out bt conventional cytogenetic study) in 3 years time and to analyze 70-80 MR individual from that cohort in this reporting period. We expect more candidate pathogenic CNVs loci to be found in this study leading to the development of a network of neurodevelopmentally-associated genes. The informed network of neurodevelopmentally-associated genes will help us understand the etiology of MR disorders and will assist in diagnosing, managing and treatment of the disorders.

文獻探討 (Literature Review)

BAC/PAC clone-based array CGH is the first to develop for screening submicroscopic deletion and duplication associated with mental retardation (Bejjani et al., 2005; Rosenberg et al., Menten et al., 2006). Some of the BAC arrays used are specially targeted for gene of interested (Bejjani et al., 2005). Later, whole-genome oligonucleotide arrays have been developed to investigate individuals with MR (Friedman et al., 2006; Peiffer et al., 2006; Fan et al., 2007). Using the Agilent 44K genome-wide oligonucleotide array, Fan and his colleagues were able to detect pathogenic imbalances in 15% of unexplained MR cases and found a small number of large-scale DNA copy-number variations (CNVs) (0.72/individual). In

addition, they observed a large number of CNVs (20/100 individual) in selected cases and their normal parents when a Agilent 224K platform was used. Four major manufacturers of oligonucleotide based array platforms for genome-wide of copy number alterations analysis are Affymetrix, Agilent, Illumina and NimbleGen. Among those, Affymetrix GeneChip array and Illumina BeadChip array also have SNP genotypes available for LOH (loss of heterogenesity) analysis and copy number validation, but require the presence of a validation SNP within the probe sequence. The Agilent CGH microarray assay compares test and reference sample in the same experiment. Both Affymetrix array and Agilent array CGH platform have been successfully used in the detection of copy number alteration in patient with MR (Friedman et al., 2006; Fan et al., 2007; Toruner et al., 2007). Such copy number variations have also been successfully detected using Illumina array (Peiffer et al., 2006) and NimbleGen array CGH platforms (Sharp et al., 2007) in MR patients. Most array platforms have background noise and the level of noise is inversely proportion to the length of the oligonucleotide probes (Ylstra et al., 2006). Thus, the Agilent (60 mers) and Nimble Gen (50-85 mers) array would be expected to have less background noise as compare to Affymetrix array with 25 mers. However, as alteration sizes approach 500 kb the Agilent 244K, Numblegene 385K and Affymetrix 500K platforms show very similar performance (Coe et al., 2007).

Since the development of aCGH and the discovery of copy number variation (CNV) in human genome (Infrate et al., 2004; Sebat et al., 2004), over a dozen studies have been reported, using different array platforms with varying degrees of resolution (ranging from 80-100 kb to over 1 Mb) to detect the genomic imbalances associated with ideopathic MR (Vissers et al., 2003; Shaw-Smith et al., 2004; de Vries et al. 2005; Schoumans et al. 2005; Tyson et al. 2005; Friedman et al. 2006; Krepischi-Santos et al. 2006; Menten et al., 2006; Miyake et al. 2006; Rosenberg et al., 2006; Fan et al. 2007). These results showed up to 10% of MR patients with apparently pathogenic CNVs. It should be noted that although a substantial number of CNVs have been detected in different cohorts of MR patients, most of these CNVs have not actually been validated to be pathogenic for MR (Shaw-Smith et al., 2006). Therefore, it is imperative that in order to identify *de novo* CNVs, associated with MR and more likely be pathogenic, CNV assays should be performed on patient-parents-trio samples for new found (Friedman et al., 2006; Sharp et al., 2006). In addition, other efforts need to be made to validate pathogenic CNV (Lee et al., 2007) for MR.

研究方法 **(Materials and Methods)**

Materials

We initially had using G-banding, FISH with microdeletion syndrome probes, MLPA and subtelomeric FISH to screen the 242 MR patients. Among those, 195 MR were known not to have numerical or structural aberration detected by conventional cytogenetic study and no microdeletion disorders or subletomeric anomalies detected by molecular cytogenetic techniques used. High quality DNA sample from those 195 MR patients had already been prepared (Using Gentra DNA purification kit, Genrea systems, Minneapolis), among those, 95 MR DNA samples were used for this proposed aCGH investigation. Fixed cell pellet from each of the above MR patents were also available for confirmation study by FISH based experiments.

Experimental Approach

For genome-wide high resolution array CGH study on the above cohort of MR patients, both Affymetrix and Agilent platform were used. We also applied florescent in situ hybridization (FISH)-based

technology for confirmation study when needed. The genomic DNA extracted from patients and from patient's parents (when needed) will be cataloged, quantity and quality determined. The following were outline of experimental approach:

(1) Array CGH with Affymetrix Genome-Wide human SNP array 6.0 chip or Agilent 244K chip were performed on each DNA sample.

(2) Data analysis will be performed using Affymetrix Genotyping Console software 3.0.1 or Agilent data analysis software.

(3) Array CGH result obtained were analysis to record any CNV and checked with CNV Database to detect novel CNV.

(4) Candidate *de novo* CNV werel subject to the following verifications: Confirmed by FISH study with proper BAC clone probes and fiber FISH (Li et al., 2000) if needed. A BAC library with over 2,800 FISH mapped BAC clones (CHORI BAC/PAC resources) is available in our laboratory to be used as probes for require FISH study.

結果與討論 **(Results and Discussion)**

Genome-wide microarray analysis was performed on DNA samples form 95 mental retardation (MR) patients. Detailed conventional and molecular cytogenetic studies had been carried out in those patients to rule out any detectable chromosome aberration. Two chromosomal microarray platforms (Affymetrix and Agilent) were used. Genomic imbalances presented as copy number changes (CNC) were detected in 14 MR patients (14/95). The diagnostic yield is 14.7%. A total of 23 chromosomal sites were found to have duplication (CNC gain) or deletion (CNC loss). The size of CNC duplication detected range from 56 Kb to 3.46 Mb and the size deletion detected range from 27.5 Kb to 5.03 Mb. At least 53 functional genes are involved in those CNC sites and 15 of those genes could be potentially associated with neuronal cell development in the brain (see Table 1). Some examples of chromosomal microarray analysis results are shown in Figure 1 to Figure 3.

A recent study comprised chromosomal microarray analysis results on patients with developmental delay (DD)/mental retardation (MR) and malformation from over 30 laboratories revealed a diagnostic yield of 15-20%. In other word, 15-20% of such patients were found to have CNC (Miller et al., 2010). A consensus statement from the study suggests that chromoaomal microarray is a first-tier clinical diagnostic test for individuals with developmental deisabilities or congenital anomalies. The result of our study showed about 15% of detection rate my microarry analysis for MR is in agreement with above study. A number of candidate pathogenic CNC loci Had been found in this study that could have future investigation of a network of neurodevelopmentally-associated development genes. Such network of neurodevelopmentally-associated genes will shed more light on the etiology of MR disorders and will assist in diagnosing, managing and treatment of the disorders.

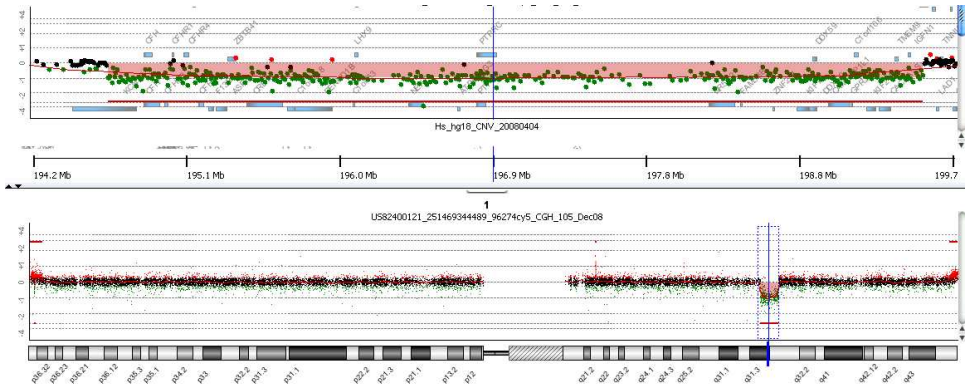
Table 1. MR cases with genomic imbalance/copy number change (CNC) detected by aCGH

Case No.	ID	CNC		Chromosomal position	Candidate gene involved	Remarks
		gain	loss			
1	96274		V	**1q31.3-32.1**: **(5.03Mb)** 194673646-199701113	KCNT2, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, F13B, ASPM, ZBTB41, CRB1, DENND1B, ATP6V1G3, NEK7, PTPRC, NR5A2, KIF14, DDX59, CAMSAP1L1, CACNA15, TMEM9, IGFN1, PKP1.	- Immunohistochemistry on rat brain showed that Kcnt2 is widely distributed throughout the brain - ASPM is crucial for maintaining a cleavage plane orientation that allows symmetric, proliferative division of neuroepithelial cells during brain development. - haplotype carrying an 84-kb deletion of the CFHR1 and CFHR3 genes (605336.0001) was associated with decreased risk of ARMD.
2	96002	V		**14q12: (0.21MB)** 24390022-24597299	STXBP6	- *De novo* STXBP1 mutations in mental retardation and nonsyndromic epilepsy have been reported.
3	96003		V V V	**8q13.1: (61Kb)** 66900992-66962393 **12q21.32: (52Kb)** 86968706-87021125 **18p11.23: (37Kb)** 8027248-8064402	PDE7A; CEP290; PTPRM; PCDH11Y	- CEP290 protein is localized to the centrosome and cilia and has sites for N-glycosylation, tyrosine sulfation, phosphorylation, N-myristoylation, and amidation. - PTPRM proteins regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. - PCDH11Y protein is thought to play a fundamental role in cell-cell recognition essential for the segmental development and function of the central nervous system
		V		**Yp11.2: (0.18Mb)** 5453514-5634276		
4	96034		V	**2q31.3-32.1: (0.27Mb)** 156697229-156966402	NR4A2	- Mutations in this gene have been associated with disorders related to dopaminergic dysfunction, including Parkinson disease, schizophernia, and manic depression.

5	96217		V V	**2p25.1: (27.5Kb)** 9442631-9470174 **22q12.3: (0.86Mb)** 32277831- 33142574	DDEF2, ITGB1BP1, LARGE	ITGB1BP1 protein plays an important role during integrin-dependent cell adhesion. - Mutations of LARGE gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation
6	96224		V V	**6q14.3: (32Kb)** 84935583-84967503 **12q21.32: (38Kb)** 85219610-85257689	KIAA1009; MGAT4C	KIAA1009 play an important role in cell division regulating chromosome segregation and mitotic spindle assembly.
7	96226	V	V	**3p24.1: (0.27Mb)** 27203623-27475123 **17p11.2: (3.46Mb)** 16698089-20160197	NEK10, NEK10, SLC4A7, SBC2 RAI1	RAI1 gene is dosage-sensitive for SMS and PTLS, is active in nerve cells the in brain. *Dp*(11)17/+ mice can be rescued by *Dp*(11)17/*Rai*1-compound heterozygous animals.
8	96231		V	**1p22.3: (52Kb)** 87166845-87219199	HS2ST1	
9	96232	V	V V	**21q21.3: (56.8Kb)** 25886714-25943467 **Xp11.1-11.2: (0.43Mb)** 56323095-56756731; **3p21.31: (0.22Mb)** 47692868-47910536 **9p24.1-24.2: (0.59Mb)** 4372284-4960293	MRPL39, JAM2, SLC1A1, CDC37L1	
10	96235		V	**1p31.1: (0.19Mb)** 74388648-74574455	LRRC44, FPGT, TNNI3K	TNNI3K may play a role in cardiac physiology.
11	96238	V		**2p11.2: (56Kb)** 85779138-85835589	GNLY, ATOH8	ATOH8 is a putative transcription factor. May be implicated in specification and differentiation of neuronal cell lineages in the brain.
12	96245	V		**16 p31.1: (0.13Mb)** 8963549-9095701	USP7, C16orf72	
13	96250	V		**18q22.3-23: (0.12Mb)** 71192127- 71308508	C18orf62	
14	96255		V	**6p22.3: (99Kb)** 20941346-21040387	CDKAL1	

del1q31.3-32.1

chr1:194673646-199701113 (~5.03 Mb)

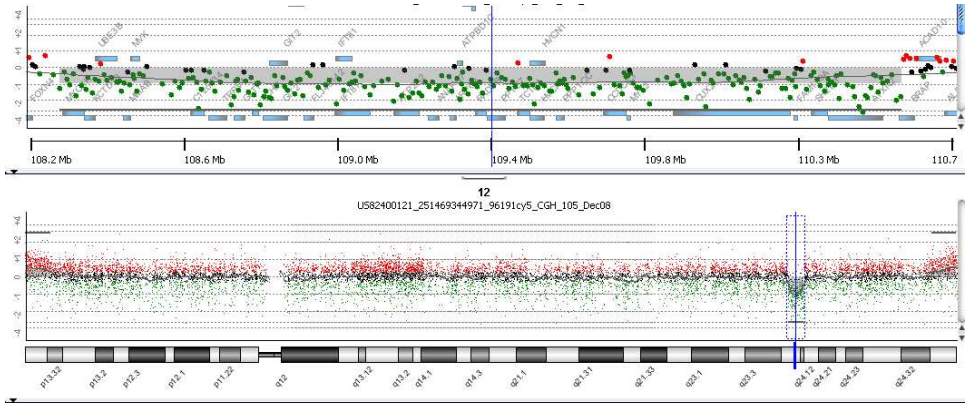


ASPM is a candidate gene for autosomal recessive microcephaly.
May be a kind of haplo-insufficiency of ASPM

Fig.1 A ~5.03 Mb deletion at chromosome site 1q32.3-32.1 (194673646-199701113) was detected in a MR patient by chromosome microarray analysis using Agilent 244 chip.
One of the functional gene in this deleted region is ASPM

del12q24.11-24.12

chr12: 108305111-110572315 (~2.27 Mb)



- MYO1H, KCTD10, UBE3B, MMAB, MVK, C12orf34, TRPV4, GLTP, TCHP, GIT2, GIT2, ANKRD13A, FLJ40142, IFT81, IFT81, ATP2A2, ANAPC7, ARPC3, ATPBD1C, C12orf24, VPS29, RAD9B, PPTC7, TCTN1, HVCN1, HVCN1, PPP1CC, CCDC63, MYL2, CUX2, FAM109A, SH2B3, ATXN2, BRAP

Fig.2. A ~2.27 Mb deletion at chromosome site 12q24.11-24.12 (108305111-110572315) was detected in a MR patient by chromosome microarray analysis using Agilent 244 chip. A number of functional in the deleted region is indicated.

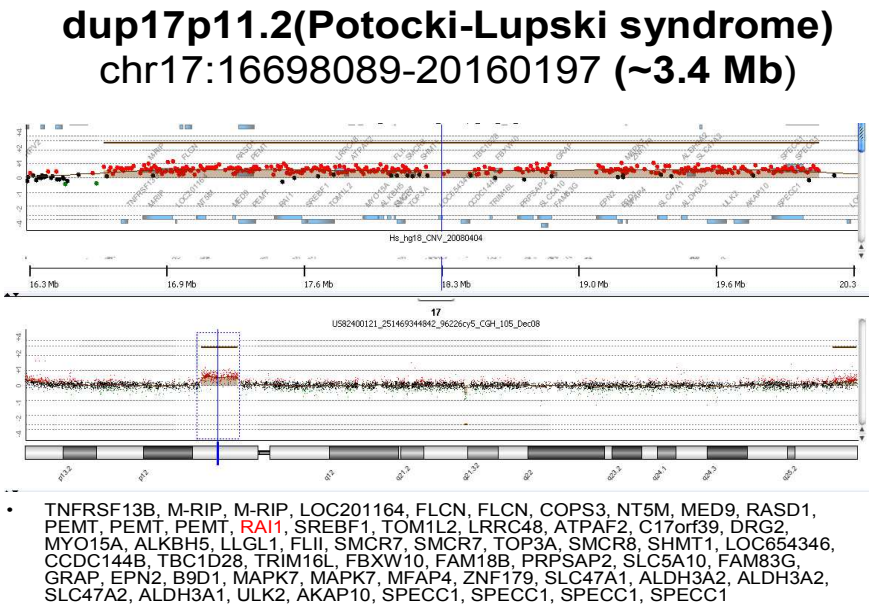


Fig.3. A ~3.4 Mb duplication at chromosome site 17p11.2 (16698089-20160197) was detected in a MR patient by chromosome microarray analysis using Agilent 244 chip. Functional genes associated in the duplicated region are shown including in candidate gene RAI1.

論文發表 (Publications)

Chien SC, Li YC, Ho M, Hsu PC, Teng RH, Lin WD, Tsai FJ, Lin CC. Rare rearrangements: A "jumping satellite" in one family and autosomal location of SRY gene in an XX male. Amer J Med Genet Part A 149A:1775-2781, 2009.

Sung PL, Chang SP, Wen Kc, Chang CM, Yang MJ, Chen LC, Chao KC, Huang CHF, Li YC, Lin CC. Small supernumerary chromosome 10 associated with an apparently normal phenotype. Amer J Med Genet Part A 149A:2768-2774, 2009.

Chien SC, Li YC, Li LH, Wu JY, Hsu P, Shi SL, Tsai FJ, Lin CC. A new familial insertion, ins(18;9)(q12.2;q33.1q31.1) with a 9q31.1-9q33.1 deletion in a girl with cleft lip and palate. Amer J Med Genet Part A 152A:1862-1867, 2010.

參考文獻 (References)

Bejjani BA, Saleki R, Ballif BC, Rorem EA, Sundin K, Theisen A, Kashork CD, Shaffer LG. Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: is less more? *Am J*

Med Genet A. 34:259-267, 2005.

Coe BP, Ylstra B, Carvalho B, Meijer GA, MacAulay C, Lam WC. Resolving the resolution of array CGH. *Genomics* 89: 647-653, 2007

de Vries BB, Pfundt R, Leisink M et al. Diagnositc genome profiling in mental retardation. Am J Hum Genet 77:606-616, 2005.

Fan YS, Jayakar P, Zhu H, Barbouth D, Sacharow S, Morales A, Carver V, Benke P, Mundy P, Elsas LJ. Detection of pathogenic gene copy number variations in patients with mental retardation by genome wide oligonucleotide array comparative genomic hybridization. *Hum Mutat*. 28:1124-32, 2007

Flint J, Wilkie AOM. The genetics of mental retardation. *British Medical Bulletin*. 52:453-464, 1996.

Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Asano J, et al. Oligonucleotide microarray analysis of genomic imbalabce in children with mental retardation. *Am J Hum Genet* 79:500-513, 2006.

Infrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of the large scale variation in the human genome. *Nat Genet* 36:949-951.

Ji Y, Eichler EE, Schwartz S, Nicholls RD. Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res.* 10:597-610, 2000.

Krepischi-Santos AC, Vianna-Morgante AM, Jehee FS et al. Whole-genome array-CGH screening in undiagnosed syndromic patients: old syndromes revisited and new alterations. Cytogenet Genome Res 115:254-261, 2006.

Lee C, Iafrate AJ, Brothman AR. (2007) Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. Nat Genet.39(Suppl):S48-54

Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet.* 14:417-422, 1998.

Menten B, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S et al. Emerging patterns of cryptic chromosomalimbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review published reports. *J Med Genet* 43:625–633, 2006.

Miller DT, Adam MP, Aradhya S et al. Consensus statement: Chromoaomal microarray is a first-tier clinical diagnostic test for individuals with developmental deisabilities or congenital anomalies. Amer J Hum Genet 86:749-764, 2010.

Miyake N, Shimokawa O, Harada N et al. BAC array CGH reveals genomic aberrations in idiopathic mental retardation. Am J Med Genet A 140:205-211, 2006.

Peiffer DA, Le JM, Steemers FJ, Chang W, Jenniges T, Garcia F, et al. High-resolution genomic profiling of chromosomal aberrations using Illumina whole-genome genotyping. *Genome Res* 16:1136-1148, 2006.

Rosenberg C, Knijnenburg J, Bakker E, Vianna-Morgante AM, Sloos W, Otto PA, Kriek M et al. Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents. *J Med Genet.* 43:180-6. 2005.

Schoumans J, Ruivenkamp C, Homberg E et al. Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridization (array-CGH). J Med Genet 2:699-705, 2005.

Sebat J, Lakshmi B, Troge J, Alexander J, Toung J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lu cito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A,

Wigler M. Large-scale copy number polymorphism in human genome. Science 305:525-528, 2004

Sharp AJ, Selzer RR, Veltman JA, Gimelli S, Gimelli G, Striano P, et al. *Hum Mol Genet* 80:938-947, 2007.

Shaw-Smith C, Redon R, Rickman L et al. Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet 41:241-248, 2004.

Toruner GA, Streck DL. Schwalb MN, Dermody JJ. An Oligonucleotide based array CGH system for detection of genome wide copy number changes including subtelomeric regions for genetic evaluation of mental retardation. *Am J Hun Genet* A 143:824-829, 2007.

Tyson C, Harvard , Locker R et al. Submicroscopic deletions and duplications in individuals with intellectual disability detected by array-CGH. Am J Med Genet A. 139:173-185, 2005.

Vissers LE, de Vries BB, Osoegawa K et al. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. Am J Hum Genet 73:1261-1270, 2003.

Wahlstrom J. Gene map of mental retardation. *J Ment Defic Res.* 34:11-27, 1990.

Winter RM, Baraitser N. (Eds) *Multiple Congenital Abnormalities: A Diagnostic Compendium.*London: Chapman and Hall, 1991.

Ylstra B, van den Ijssel P, Carvalho B, Brakenhoff RH, Meijer GA, BAC to the futurel or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res* 34:445-450,

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

99 年 9 月 28 日

附件三

報告人姓名	林齊強	服務機構及職稱	中國醫藥大學附設醫院醫學研究部教授
會議 時間 地點	99 年 9 月 21 日至 24 日 法國馬賽	本會核定補助文號	NSC98-2314-B-039-003
會議名稱	(中文) 第 14 屆演化生物學會議 (英文) 14th Evolutionary biology meeting		
發表論文題目	(中文) 利用跨物種 BAC-FISH 和 BAC-end 序列精密描述印度山羌和人類及牛類之間的比較基因體圖譜 (英文) **Fine delineated comparative genome mapping between Indian muntjac-human-cattle by cross-species BAC-FISH and BAC-end sequencing**		

報告內容應包括下列各項：

一、參加會議經過:

第一天下午辦理報到及海報的張貼，中餐與來自法國的研究學者做了學術交流，飯後聽了兩場有關比較基因體學的演講，晚餐則與來自英國及德國的學者做了許多學術及文化交流。

第二天早上聽了一場有關癌症細胞的微小演化論。中餐與來自拉脫維亞的研究學者做了學術交流及文化交流，下午聽了兩場如何利用生物資訊探討 DNA 結構，比較同源性 DNA 片段及影響人類壽命長短主因的演化論，還有和人類有的的多基因及單基因疾病的演化速度差異。

第三天中餐與來自德國的研究學者做了學術交流，下午聽了如何利用第二代定序組裝重複性的微小衛星 DNA 序列。晚餐與來自拉脫維亞的學者做了學術交流談論有關癌症細胞染色體數目可能由多套數變成雙套數的假說.

第四天觀賞海報及海報展示說明。中餐與來自中國的研究學者做了學術交流談論有關如何以物理現象解釋基因組大小與基因大小的演化機制。

二、與會心得:

The Evolutionary Biology Meeting at Marseilles is an annual event which has gathered high level expertise in the field of evolutionary biology since its creation in 1997. This year's Annual Meeting at Marseilles has reached a worldwide dimension and plays a paramount role in the international scientific community: allowing the gathering of high level specialists to exchange their ideas and to stimulate the way of thinking on evolution in their field of works in different parts of the world.

This is my first time to attend this meeting. This meeting is different from the other "Big international meeting" such as ASHG annual meeting I usually attended. Although this meeting is smaller than ASHG annual meeting, it benefits having more chances to discuss with the researchers who are the experts in the field by face to face. In addition of scientific exchange, I have a lot of chances to chat with culture issue with colleagues who are from different countries during this meeting. It let me award of different environment of research, funding problems and culture in different countries.

Appendix： 壁報論文

Fine delineated comparative genome mapping between Indian muntjac-human-cattle by cross-species BAC-FISH and BAC-end sequencing

C.C. Lin[1], T.-S. Li[2], P.-C. Hsu[1,2], Y.C. Li[2]
1. Department of Medical Research, China Medical University Hospital, Taichung, Taiwan.
2. Department of Biomedical Sciences, Chung-Shan Medical University, Taichung, Taiwan.

Indian muntjac (*Muntiacus muntjak vaginals*) is a unique mammalian species with 2n=6/7 (the lowest chromosome number in mammals). The chromosome of this species was thought to have evolved mainly through extensive tandem fusions and 3 Robertsonia translocations by recent molecular cytogenetic studies. The correspondent conserved segments in Indian muntjac-human-cattle were also identified by reciprocal cross-species chromosome painting (Frönicke and Scherthan 1997; Yang et al. 1997). Because the chromosome painting limits the resolution, the gene order alteration and breakpoint during karyotypic evolution is not clearly elucidated so far. The cross-species BAC FISH was used in this study for comparative mapping Indian muntjac and human. 353 of Indian muntjac BAC clones presented positive FISH signals on the human metaphase chromosomes (Figure 1 for examples) and 77 of RP11 human BAC/PAC clones had positive FISH signals on the Indian muntjac metaphase chromosomes (Figure 2 for examples). 27 interstitial intervals each in the haploid chromosome of Indian muntjac homologized to single synteny of human genome excepting 1p41-1p34 interval with two homologous segments from HSA1 and HSA2, 1p22.1-1p17 interval having HAS 17 and HSA22q12.1, 1q17-1q21 interval detected with HSA4, 12 and 22, 1q32-1qt interval equivalent to HSA19p and HSA5, 2p region corresponding to the HSA12/22, 2q13.2-2q2q15 interval and 3q41-3qt interval both having HSA14/15, 2q26-2q34 interval homologizing to HSA16 with HSA17 interrupted and HSA19, and 2q35-2q36 interval with HSA10-interrupting HSA20. A total of 44 conserved synteny segments were identified between Indian muntjac and human (Figure 3). The othologous human gene order in some conserved synteny segements is also identified (Figure 4). Furthermore, the sequence similarity of Indian muntjac BAC-end sequences (BESs) to human and cattle was performed by BLASTN. Among 957 high quality BESs, 265 BESs significantly hit to the human specific loci and 800 BESs anchor to the cattle chromosome with $\geq$ 200 bp and $\geq$ 70% identities (E $\geq$ e-7). A comparative genome map in Indian muntjac, human and cattle is renew in more high resolution by cross-species BAC-FISH and BLAST hit of BESs. The map showed that 27 interstitial satellite signal intervals in the haploid chromosome of Indian muntjac each correspond to one homologous BTA segment with the exception of 1p22.1-1p17 interval containing two syntenies from BTA17 and BAT19, 2q13.2-2q15 interval having BTA10 and BTA21, 2q24-2q25.3 interval corresponding to BTA28 and BTA26, 2q26-2q34 interval with BTA 25 and BTA 18, while 1pt-1p43 and 1p43-1p42.1 intervals only having BTA11, 3q37-3q38 and 3q38-3q39 intervals homologizing to BTA 6. A total

of 37 conserved segments were identified between Indian muntjac and cattle. Most of cattle chromosomes each maintained an entire synteny excluding BTA1, BTA2, BTA5, BTA8, BTA9, BTA10, and BTA17(Figure 3). Taken our result and the previously published data together, the evolutionary comparative map between pecoran ancestor, Cetartiodactyl ancestor, human, cattle, and muntjac is constructed in this study (Figure 5). According to this evolutionary comparative map, the evolutionary breakpoints are predicted during the evolution from Cetartiodactyl ancestral karyotype (CAK) to pecoran ancestral karyotype (PAK) and from PAK to Bovidic karyotype and Cervidic karyotype (Table 1). Additionally, the conserved gene order in some synteny blocks is clarified.

ACKNOWLEDGEMENT

We acknowledge the grant supports from the National Science Council NSC97-2311-B-040-003-MY3 and NSC98-2314-B-039-003

FIGURES AND FIGURE LEGENDS:

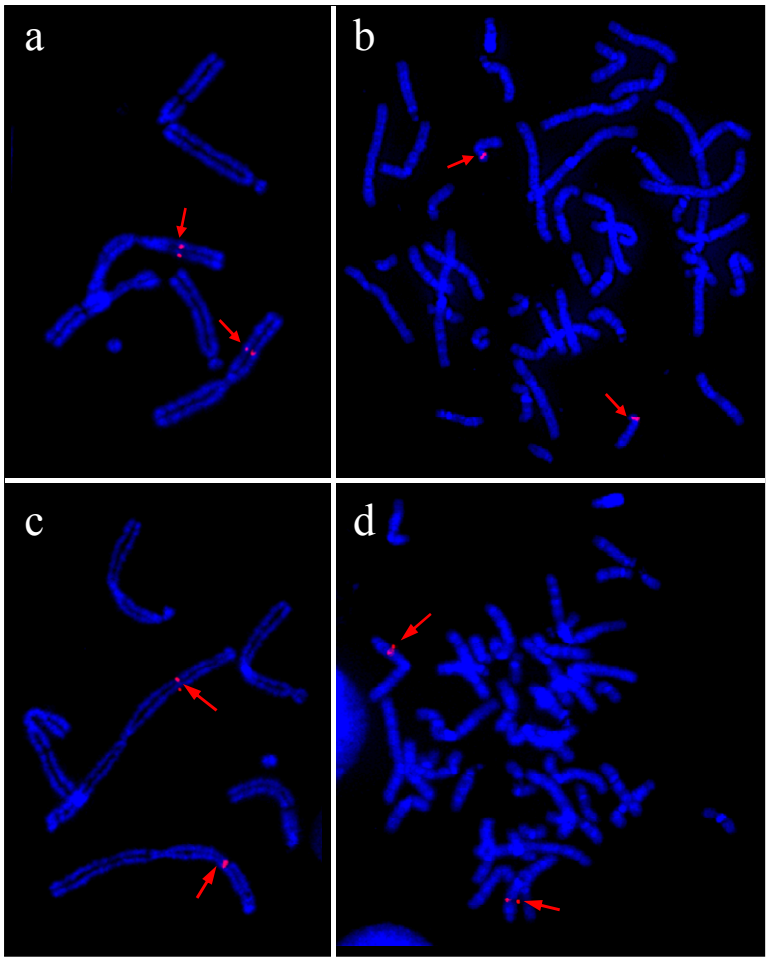


Figure 1: The result of cross-species BAC-FISH. About 2-3 Indian muntjac BAC clones for each G-band of Indian muntjac ideogram were selected and mixed to map onto the human metaphase chromosomes. A mix of IM04-197E6 and IM04-196A1 on the 1p23 of the Indian muntjac chromosomes (a) was mapped onto the 18p11.2 of the human metaphase chromosomes (b). A mix of IM04-219A1, IM04-307H12 and IM04-526B9 on the 1q23 of the Indian muntjac chromosomes (c) was mapped onto the 3p21 of the human metaphase chromosomes (d).

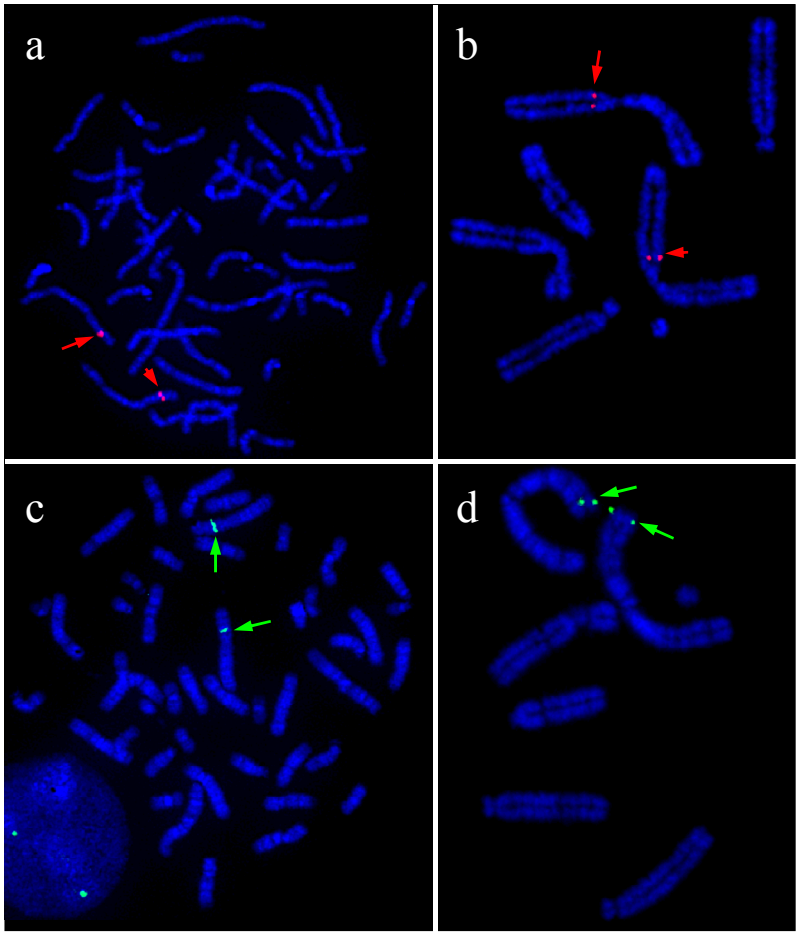


Figure 2: The result of cross-species BAC-FISH. About 2-3 human RP11 BAC clones for each G-band of human ideogram were selected and mixed to map onto the Indian muntjac metaphase chromosomes. A human RP11 BAC DNA mix of 3q26.1 (a) was mapped onto the 1q14 of the Indian muntjac chromosomes (b). A human RP11 BAC DNA mix of 2p16 (c) was mapped onto the1p43 of the Indian muntjac chromosomes (d).

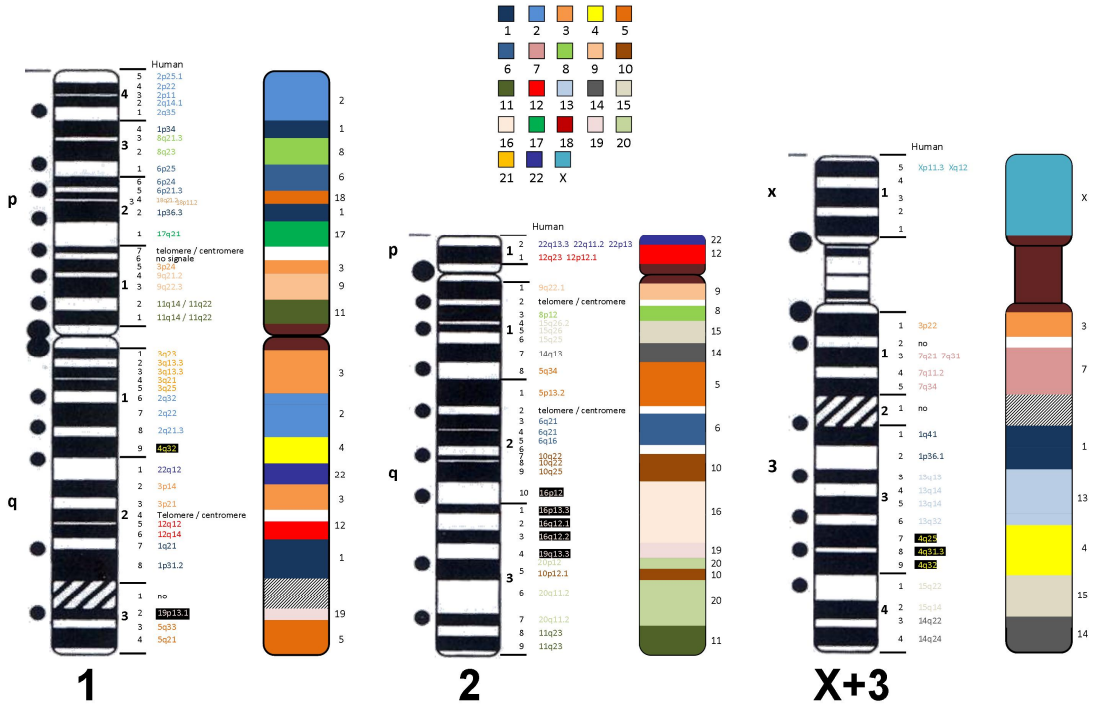


Figure 3: A comparative map of Indian muntjac to human was constructed by summarizing the result of cross-species BAC-FISH that 88 mixes of muntjac BAC DNA was mapped onto the human metaphase chromosomes. The black-white ideogram represents the karyotype of Indian muntjac (Li, et al. 2000). The color ideogram of Indian muntjac karyotype demonstrates segmental homology with human chromosomes and the corresponding bands between Indian muntjac and human were indicated in the middle of color and black-white ideogram. The black circles represent the localization of interstitial satellite I.

表 Y04

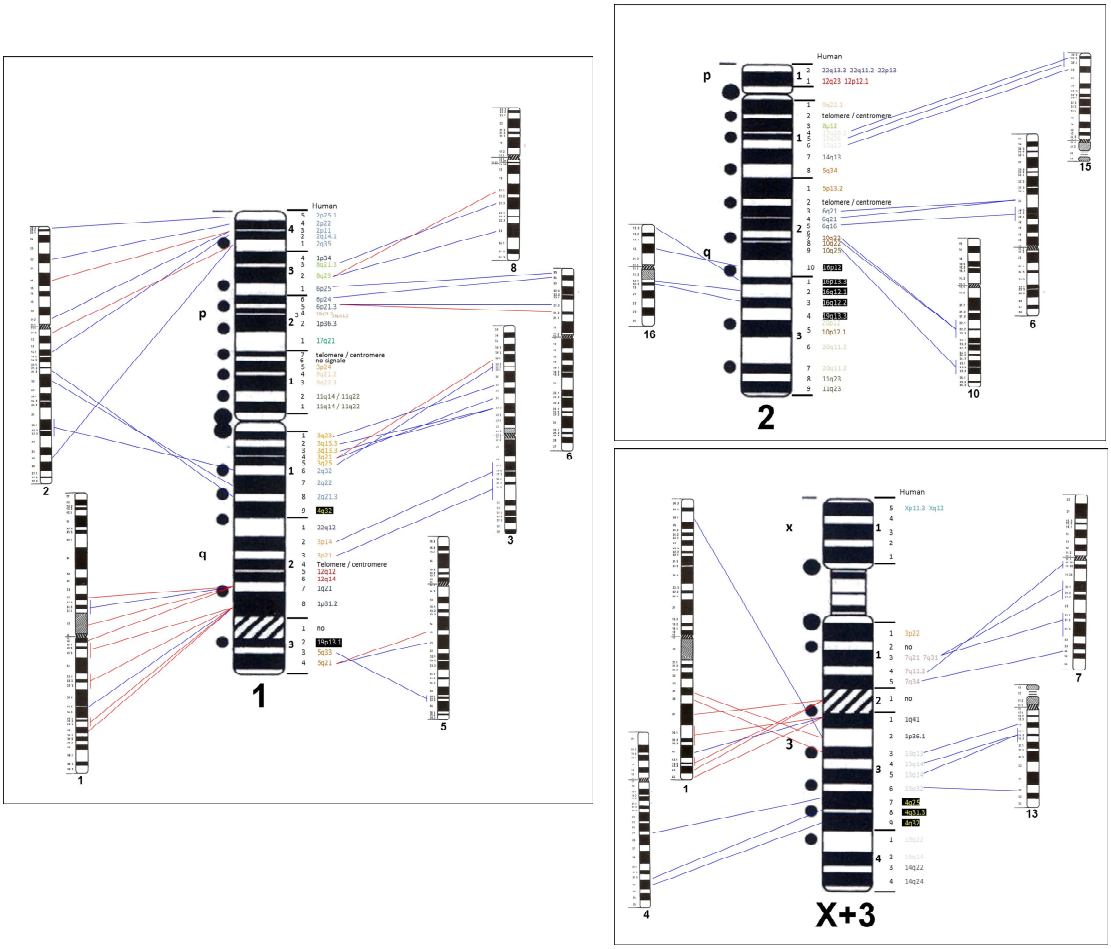


Figure 4: Summary of the conservation of gene orders between Indian muntjac and human. Small sized human ideograms were along two sides of the big sized Indian muntjac ideogram. More than 3-adjoining bands of Indian muntjac with homologous segments of the same human chromosome were indicated. The blue lines represent the result of Indian muntjac to human cross-species BAC-FISH. The red lines represent the result of human to Indian muntjac cross-species BAC-FISH.

表 Y04

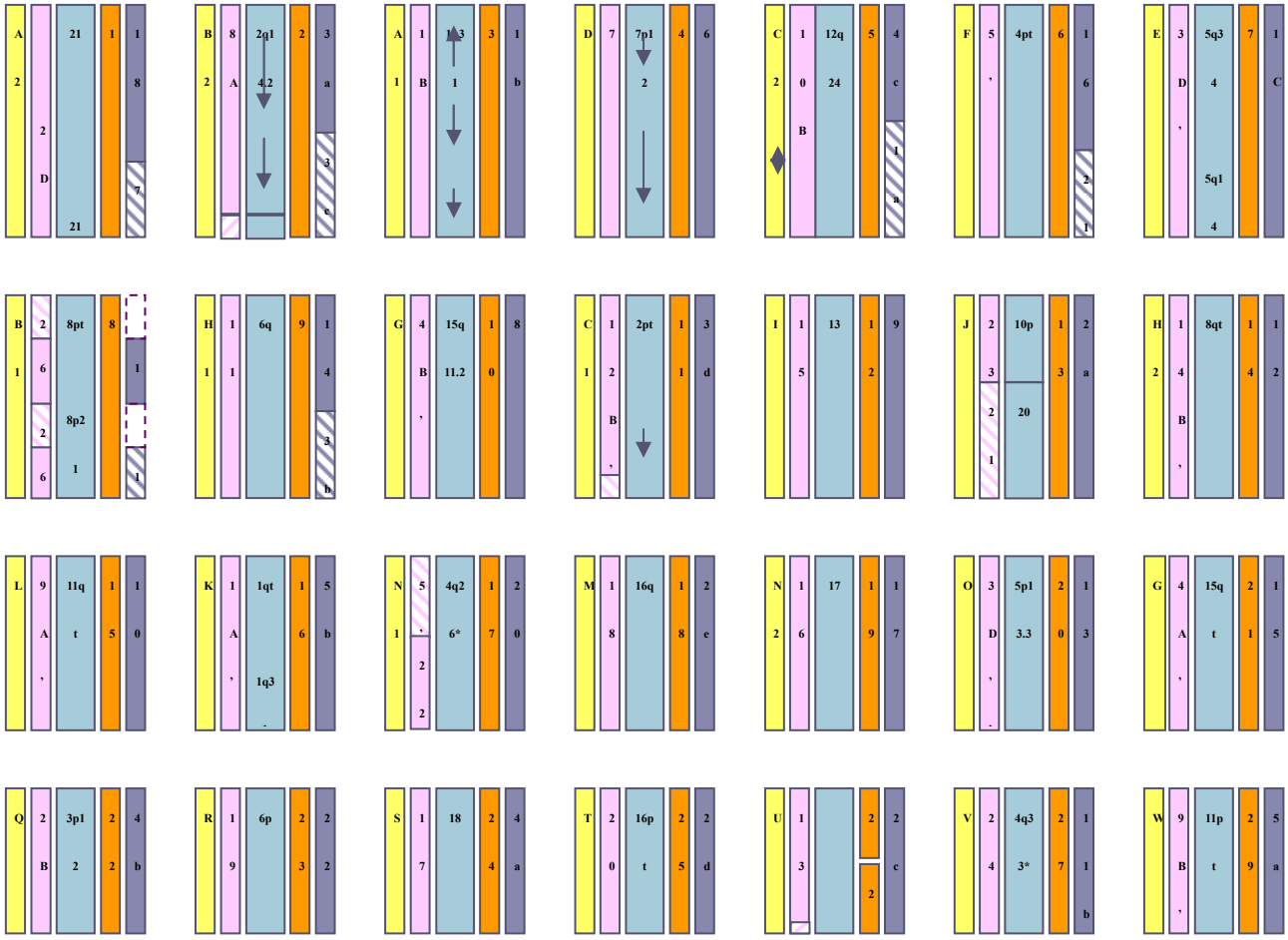


Figure 5: The evolutionary comparative map between pecoran ancestor, Cetartiodactyl ancestor, human, cattle, and Chinese muntjac. This map is lined by cattle chromosomes.

表 Y04

國科會補助計畫衍生研發成果推廣資料表

日期:2010/11/17

國科會補助計畫	計畫名稱：台灣智能障礙族群基因體失衡之研究
	計畫主持人：林齊強
	計畫編號：98-2314-B-039-003- 學門領域：小兒科

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：林齊強	計畫編號：98-2314-B-039-003-
計畫名稱：台灣智能障礙族群基因體失衡之研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	1	1	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本研究成果發現利用染色體微晶片(chromosomal microarray)技術對智障者基因體失衡之偵測率(diagnostic yield)可到達 15%，此項初步研究結果與國外最近研究報告相當符合(Miller et al., 2010 Amer J Hum Genet 86:749-764)。在未來決定首先採用染色體微晶片(chromosomal microarray)技術對於先天性智能發育遲慢者如智障，自閉症等之疹診斷將有重要貢獻。而且在基因體失衡之區域中存在若干重要基因，未來對這些基因功能之研究可能探討是否與智障致病之機制有相關性。」