附件四、技術說明表

發掘可差異性診斷巴金森症候群病人之功能性血漿 microRNAs 與胞泌體蛋白質生物標記的高效數據分析套裝方案

提案人： 林劭品 教授
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簡　歷：(可列出相關連結，例如系所、研究室網頁)
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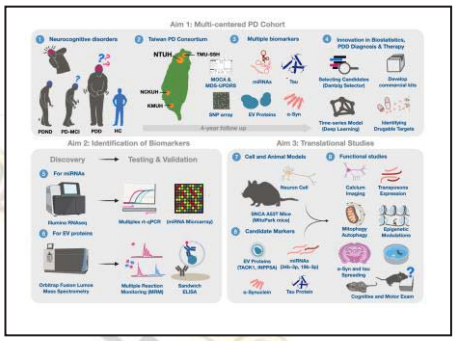

市場及需求:

巴金森氏病是 65 歲以上第二常見且不可逆的神經退化性疾病，除此之外也會造成非動作症狀如輕度認知障礙及失智症，目前巴金森氏病合併失智症缺乏機轉相關的生物指標，且現存治療巴金森氏病的藥物無法減緩其症狀，甚至會對藥物產生嚴重副作用，因此早期區分出巴金森症合併認知退化的方法及治療藥物仍是未被滿足的醫療需求。血中微型核糖核酸(microRNA)與細胞外泌體(EV)蛋白能作為早期診斷的生物標記，並且具有發展商品化檢測套組的潛力，因此我們希望找出有力的生物標誌物，進一步發展新型巴金森氏病檢測技術，以改善對此類非典型巴金森氏病的臨床需求。

技術摘要(含成果):

技術包含從病人臨床診斷及血漿抽樣方法，將病人依不同病徵分群，到分析病人血漿中游離微型 RNA 與胞外泌體蛋白表現，開發深度學習之統計方法 BOLD Selector，找尋精準治療的生物標記。對於臨床診斷和血漿採樣，我們按照統一的方案將患者分為不同的組，PDD(PD 合併失智症)、PD-MCI(PD 輕度認知障礙)、PDND（PD 正常認知功能）、MSA-C（多重性神經退化症 C 型）、MSA-P（多重性神經退化症 P 型）和 HC（控制組）。利用高通量小型非編碼 RNA 定序及傅立葉轉換電場軌道阱複合式質譜儀分析患者血漿中的 microRNA 與 EV 蛋白，接著通過改良的 BOLD Selector 演算法篩選生物標記，找到候選生物標記。在我們的初步研究中，我們已經確定了血漿中數種 microRNA 和 EV 蛋白，作為這些巴金森氏病(包括具有認知障礙的 PD)的生物標誌物，並且基於這種分析方案能計算出 logistic regression formula 來建立預測模型，之後可作為判斷 microRNA 和 EV 蛋白一類之生物標記是否能有效區分巴金森亞型的依據。

優勢:

病人臨床診斷及血漿抽樣方法的統一，減低過程中的變異並確保樣品的保存，利用次世代基因定序和質譜儀大量分析出病人血漿中游離微型 RNA 與胞外泌體蛋白的表現量，BOLD Selector 演算法經過創新客製並優化，為精確篩選出病人血漿中與認知功能變化相關的游離微型 RNA 與胞外泌體蛋白，這種方法可以從

大量資訊中快速且精確地找到有效的生物標記，也能在許多其他基於臨床樣本的生物標誌物鑑定中實施。生物標記與臨床檢測試劑能彌補巴金森症病人早期診斷的醫療需求，透過生物標記將病人分類，使病人能及早對症下藥。研究結果將提供巴金森病產生失智症之生物指標，進行預防與治療，降低社會醫療負擔，提升我國在該領域的國際聲譽。

競爭產品:

專利現況:

(1) 本技術已有（申請）相關美國臨時專利，application number 63455953

(2) 吳瑞美醫師在巴金森氏病的臨床遺傳學、認知和病理生理學等研究方面享有盛譽和經驗，與郭明哲醫師致力於神經多重退化症以及神經心理學研究，以發展完整診斷病患之方法，並協助巴金森患者血漿收案。林劭品教授是研究功能性 RNA 和表觀基因組調控、生物資訊學分析以及細胞生物學的專家，於微型 RNA 有多年研究經驗;蔡億蒼博士有多年研究神經生物學的經驗及實驗能力，和林教授一同前往日本與研究游離微型 RNA 與胞外泌體蛋白的專家落谷孝広教授和植田幸嗣教授進行合作，成功地分析了巴金森氏病合併認知障礙患者和對照組的血漿微型 RNA 和血漿胞外泌體蛋白。在大量微型 RNA 和血漿胞外泌體蛋白的數據被分析出來後，與潘建興教授合作篩選具有分類病患意義之生物標記，潘教授擁有化學、統計學、機器學習、應用數學、人工智能背景，是國際知名的數據科學家，他開發了許多新穎的方法和工具，為各個領域做出了重大貢獻，其學生黃靖雯為清大統計所博士生，專精於統計學，在篩選生物標記過程中，提供許多的統計學上的幫助。許家郎為台大醫院醫學研究部研究員，亦為統計學提供許多幫助。林延翰、許雅方為已畢業碩士生，林王象軒為在校博士班學生，亦為此專利做出貢獻。

(3)其他…

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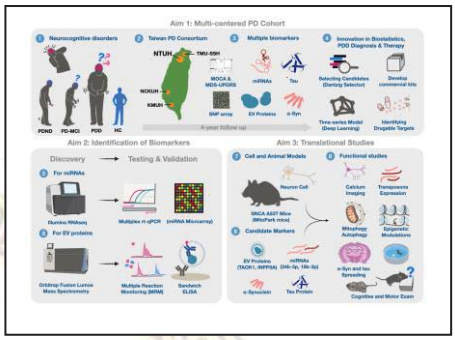
Efficient and effective profiling and data analytics scheme for identifying functional plasma microRNAs and extracellular vesicle protein biomarkers for differential diagnosis of Parkinsonism

PI： Prof. Shau-Ping Lin
Institute of Biotechnology, National Taiwan University.

Experience:

https://www.iob.ntu.edu.tw/zh_tw/faculty/full/-%E6%9E%97%E5%8A%AD%E5%93%81-68842655



Market Needs:

Parkinson's disease (PD) is an irreversible, 2nd common neurodegenerative disease in the elderly more than 65 years old resulting in both motor complications and non-motor symptoms (NMS), such as mild cognitive impairment (PD-MCI) or dementia (PDD) as neurocognitive disorders (NCD), in the advanced stage. Currently, PD with cognitive impairment is lacking biomechanism-relevant biomarkers and is badly respond to PD accompany with serious side effect, so efficient differential diagnosis and therapeutic drugs for NMS of PD are still unsatisfactory. Increasing evidence suggests that circulating plasma microRNAs and extracellular vesicle (EV) proteins related to the pathophysiology of PD can be developed into efficient analytical and commercializable kits for early diagnosis of PD with cognitive impairment and predict the progression and drug-related psychiatric complications. Therefore, we would like to identify practical and robust biomarker of microRNAs and extracellular vesicle (EV) proteins for improving clinical need for such atypical parkinsonism.

Our Technology:

Technologies include clinical diagnosis and plasma sampling, grouping subtype PD, analyzing level of circulating plasma microRNAs and extracellular vesicle (EV) proteins expressed in patients' plasma, and development of statistical method, BOLD Selector, and principal components analysis (PCA), for identifying novel plasma miRNA and EV proteins. For clinical diagnosis and plasma sampling, we classified patients into different group with unified protocol. In our datasets, the response represents the status of patients, which can be PDD (PD with dementia), PD-MCI (PD with mild cognitive impairment), PDND(PD no dementia), MSA-C(Multiple system atrophy C type), MSA-P(Multiple system atrophy P type), and HC (health control). We performed a high-throughput small non-coding RNA-seq and the Orbital Fusion Lumos and Orbital Fusion Lumos-FAIMS LC/mass spectrometry (MS)/MS to analyze circulating plasma microRNAs and extracellular vesicle (EV) proteins in patients' plasma. For identifying practical and robust biomarker, we select candidates by a novel stepwise combination of BOLD Selector mediated statistical finding of candidate microRNAs and EV proteins. In

our preliminary study, we have identified novel plasma miRNA and EV proteins as biomarkers for these Parkinsonism including PD with cognitive impairment as well as validated accuracy of classifying patients by logistic regression, allowing us to construct a prediction model for novel plasma miRNA and EV proteins.

Strength:

The unification of clinical diagnosis and plasma sampling reduces the variation in the process and ensures the preservation of samples. Via next-generation gene sequencing and mass spectrometer, plasma miRNA and EV proteins in the patient's plasma are extremely screened out. We select candidates by a novel optimized BOLD Selector. This method can handle datasets with small number of observations and large candidate factors. The methodology can be implemented in many other clinical-sample-based biomarker identifications. These robust biomarkers can improve unmet medical needs of early and differential diagnosis for PD patients with mild cognitive impairment or dementia. These results will provide prevention and treatment for PD patients, which can reduce the medical burden and enhance Taiwan international reputation in this field.

Competing Products:

Intellectual Properties:

(1) USA provisional patent, application number 63455953

(2) Prof. Ruey-Meei Wu has an excellent reputation and experience in the research of PD including clinical genetics, cognition, and pathophysiology of Parkinson's disease, worked with Dr. Ming-Che Kuo to study multiple system atrophy and neuropsychological phenomenon in patients for developing more complete diagnosis for patients, and collected plasma samples from Parkinsonism patients. Prof. Lin is the expert in studying functional RNAs, epigenomic modulation, bioinformatic analysis, and the cell biology. She has experience on studying microRNA for more than ten years. Dr. Yi-Tzang Tsai has extensive experiment on studying neurobiology. He went to Japan with Prof. Lin to cooperate with Prof. Takahiro Ochiya and Prof. Koji Ueda, the experts in studying small RNA and EV proteins, successfully profiled plasma small RNA sequences and plasma EV proteins from PD patients with different cognition abilities and control groups. After large data of plasma small RNA and plasma EV proteins came out, Prof. Frederic Phoa was invited by Prof. Lin to help with selecting candidate biomarkers for significantly distinguishing different type of PD patients. With Chemistry, Statistics, Machine Learning, Applied Mathematics, Artificial Intelligence background, Prof. Phoa is an internationally well celebrated data scientist who has developed many novel methodologies and tools, making significant contributions to various fields. His student, Jing-Wen Huang, is PhD

student of Institute of Statistics in NTHU. She is the expert in statistics, who provided a lot of statistical help in the process of screening biomarkers. Chia-Lang Hsu is a researcher at National Taiwan University Hospital's Medical Research Department, and he also provides considerable assistance in statistics. Yan-Han Lin and Ya-Fang Hsu are graduated master's students, while Hsiang-Hsuan Lin Wang is a current doctoral student and has also made contributions to this patent.

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