研究成果報告

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計畫名稱：卡介苗嚴重副作用與嚴重分枝桿菌幼兒病患之基因診斷研究

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目　　錄

貳、計畫中文摘要及關鍵詞

研究目的：卡介苗(Bacille Calmette-Guérin)為一個減毒性疫苗，是目前臨床上唯一用來對抗結核菌的疫苗。這個減毒性疫苗的使用在全球已有四十年以上歷史，安全性非常高。即使疫苗本身屬於低致病力，但已知在少數的病人身上仍會造成卡介苗感染的問題，機率大約是新生兒接種之萬分之一，台灣地區在2005 至 2008 年至少有 14 個病例被通報。近期研究顯示，重症型的卡介苗感染，可能跟幼童帶有免疫缺失有關，這樣的幼童即使不打卡介苗，也可能遭受其他的感染問題，而這些免疫缺失的病童更無法以臨床檢驗被發現。”疫苗安全性”一直以來都是社會與健康非常重要的議題，因此卡介苗感染進而變成公共衛生中受到爭議的一環。本研究目的在探討，台灣地區因卡介苗疫苗造成副作用與病人基因的關係，目前已知有超過 10 種以上單基因免疫缺失疾病會造成 BCG 或是分枝桿菌感染而無其他明顯症狀，也無法以現有的臨床檢測來診斷。我們試著對卡介苗感染或是嚴重型 TB 感染的幼童以分子醫學診斷，了解病人是否具有遺傳免疫缺失。

研究方法：我們共募集了 7 位重症型卡介苗感染與 1 位 TB 感染的幼童，收集的血液樣本進行 IL-12/IFN-γ 功能檢測、自由基產生能力與 NF-kappaB 功能反應檢視。並從病人的周邊血單核細胞中抽取得 DNA 與 RNA，若在初步的功能性檢測中發現異常，將進一步定序相關的目標基因。

結果：所有的病人對 IL-12/IFN-γ 功能檢測與 TNF 刺激反應皆正常，因此排除了 IL-12/IFN-γ與 NEMO 相關的基因缺失。另外將所有病人針對 ISG15 基因進行定序後也無發現基因缺失。然而，發現其中一位病人(BCG8)產生自由基的能力嚴重受損，定序相關基因 CYBB 後，發現是一種帶有基因變異的罕見慢性肉芽腫疾病(CGD)，結果顯示病人具有半合子缺失 665A>G (H222R)，此基因缺失已被報導與感染性疾病有相關。調查其家族史，發現此病人曾經有二位哥哥也遭受類似的嚴重細菌性感染並相繼在 2 歲前死亡。幸運地，目前此病人已找到配對合適的臍帶血細胞並進行移植手術，目前已不再遭受嚴重感染的問題。

結論與討論：我們的研究成果，可以說明宿主的遺傳免疫缺失可以解釋部分的卡介苗感染。在 CGD 患者中，化膿性細菌感染是最明顯的臨床徵狀。然而，CYBB 基因缺失已知和非結核分枝桿菌感染(NTM)有相關，且接種卡介苗可能導致嚴重卡介苗感染，也被當作是台灣在早期接種卡介苗疫苗時的重要指標。我們並未發現病人具有 IL-12/IFN-γ 缺失，可能原因為文獻大多顯示此缺失通常在 NTM 感染患者中被發現。我們也遇到招募患者的困難點，因此無法得到足夠的樣本數量來得出最終的結論。然而，我們的結果也顯示正常兒童與因接種卡介苗疫苗而產生嚴重副作用的幼童相比，可能反映出有淺在的基因缺失導致影響了抗分枝桿菌的能力。這表示在台灣受到卡介苗感染的族群中仍有相關的未知基因缺失未被發現，我們正進行外顯子全基因定序，結果仍在持續進行中。

關鍵詞：卡介苗, 分枝桿菌感染, 疫苗，幼兒

參、計畫英文摘要與關鍵字

Aim of studying: Bacillus Calmette-Guérin (BCG) is an attenuated vaccine and is only vaccine approved in clinical to against Mycobacterium Tuberculosis (TB). BCG has used more than 40 years in world-wide with very good safety record. Even low pathogenic ability, BCG infection was observed few cases; the prevalence was around 1 in 10,000 newborns vaccinated. In Taiwan, there were at least 14 cased with BCG infection had been record from 2005 to 2008. Recent studying suggested the non-pathogenic BCG infection in children might reflect the immuno-compromised due to the inborn genetic defects. Even without BCG vaccination, those children might still suffer from other infection due to their genetic immunodeficiency. Current clinical diagnosis is not able to identify the possible genetic disorders. ”Vaccine Safety” is a very important society and health issue; the BCG infection due to the vaccine became a critical debate in public health. The aim of this studying is to identify the relation the host genetic factor and BCG infection. There are more than 10 mono-genetic disorders associated BCG or mycobacterial infections in otherwise health patients, which couldn’t be diagnosed by current clinical tests. We tried to identify the possible immunogenetic defect by the molecular diagnosis in the children with BCG infection or severe TB infection in Taiwan.

Material and Method: We had enrolled 7 children with severe BCG infection and 1 with TB infection. Blood sample were tested for the IL-12/IFN-γ pathway, the production of free radical and the response to TNF stimulation. DNA or RNA sample were prepared from patients’ PBMC. Sequencing of candidate genes were performed if patients were showed to have the defect in functional tests.

Result: All patients were response well to IL-12/IFN-γ tests and TNF stimulation, which ruled out the genetic defect in IL-21/IFN-γ pathway and NEMO deficiency. ISG15 were sequenced in all patients and no mutation was found. However, one patient (BCG8) was showed an impaired response in the free radical production. Sequencing of CYBB, which was the morbid gene of chronic granulomatous disease (CGD), showed patient carried a hemizygous mutation 665A>G (H222R), which had been reported to associate with infectious diseases. Patients had two old brothers who had severe infection and were died before 2 years old. Patient were received the HLA-matched cord blood cells transplantation. He had no severe

infection after that.

Conclusion and Discussion:

Our data suggested the primary immunodeficiency might explan a part of suscepbitlity to BCG. In CGD patients, the pyogenic bacterial infection was the most dominant clinical problem; however, CYBB had showed as the genetic defect associated with non-tuberculosis mycobacterial infection (NTM) and severe BCG infection might severe as the major sign of infection due to the early BCG vaccination in Taiwan. We didn't find the patients with defect in IL-12/IFN-γ which was the most frequent cause of NTM infection in children in literature. We encountered the difficulty for the patient recruitment and we didn't reach the sufficient sample size to draw a final conclusion. However, our result showed that severe side effect of BCG infection after vaccination in otherwise normal child might reflect an intrinsic defect in anti-mycobacterial immunity gene. It is possible that some unknown genetic defect might specific associate with BCG infection in Taiwan population, and the whole exome sequencing were performed and the results are pending.

keywords：Bacillus Calmette-Guérin, mycobacterial infection, vaccine, Infants

肆、本文

1. 前言

(1) 卡介苗接種政策

卡介苗(Bacillus Calmette-Guerin, BCG)是目前世界上唯一核准用來對抗結核菌(TB, Mycobacterium tuberculosis) 感染的疫苗，是由 Mycobacterium bovis 所製成的減毒疫苗(1)。目前世界上超過 40 億人接受過此疫苗。由於過去研究中，卡介苗可以有效預防小兒肺部以外的結核菌感染(extrapulmonary tuberculosis)，因此本疫苗被列入世界衛生組織(WHO)的疫苗計畫中(2)。不過卡介苗在預防成人的結核菌感染的效果則不是那麼明顯，尤其在對肺部結核感染，世界各地的研究的結論從卡介苗僅有 0 到 60%保護效果(3)。而這樣的差異性，可能跟人體的遺傳與環境因素有關。近期的研究顯示，在接受卡介苗注射前受到的環境中分枝桿菌感染可能會影響卡介苗的保護效益(4)。另外，使用不同的卡介苗菌株準備方式可能也會影響疫苗的效力。台灣地區由於屬於高 TB 流行區域，目前衛生政策中，台灣新生兒均在出生後一個月內接種卡介苗。

(2)卡介苗造成感染問題

卡介苗是一個減毒性的活疫苗，因此卡介苗的副作用與致感染性是一個很重要的問題。長期的研究證明卡介苗是一個相當安全的疫苗。在副作用方面，

幼兒接受介苗注射後，大部分人會在注射處形成紅腫，可能伴隨低燒與不適。在兩個月內傷口會開始化膿結痂，留下長期的疤痕。這樣的過程與 BCG 再注射處繁殖並與人體免疫系統反應有關。但在少數接受卡介苗的幼兒身上則會有嚴重感染與副作用，可以觀察到注射處長期紅腫，近處的淋巴腫大，甚至更嚴的的會有近處感染造成骨髓炎(Osteomyelitis or Osteitis)或是瀰漫性(disseminated)卡介苗感染，其中瀰漫性 BCG 感染甚至會造成死亡，這些嚴重的不良反應大約發生在萬分之一到一百萬分之一左右(5)。這些卡介苗的嚴重副作用被認為與宿主免疫能力無法有效控制卡介苗菌有關，可能起因於疫苗菌株的不同，疫苗製備的問題或是宿主免疫力的問題。類似的觀察，卡介苗在 HIV 感染的幼兒中造成的嚴重副作用率極高，卡介苗不建議給予具有 HIV 感染的幼兒，就算是它們對於結核菌是有高感染問題。有家族遺傳性免疫缺失的的幼兒同樣的也不建議使用卡介苗(6)。台灣地區目前因卡介苗造成感染為一個嚴重的公衛問題，每年因卡介苗感染是藥物安全基金主要的給付項目之外，相關的負面新聞亦會造成社會對疫苗政策的負面觀感，因此這是一個急需解決的問題。

(3) 卡介苗感染的分子機制與文獻探討

(一)單基因免疫缺失與分枝桿菌感染 (Mendelian susceptibility to Mycobacterial infection)

BCG 與結核菌同屬於分枝桿菌(Mycobacteria)。分枝桿菌為環境中常見的微生物，包含 BCG 在內，大部分分枝桿菌對人體來說都是屬於低病原性。高病原

性的分枝桿菌如 M. tuberculosis, M. leprae, 與 M. ulcerans 等則會造成結核病，痲瘋與 Buruli ulcer 等的感染。不過僅有一小部接觸到這些高病原性分枝桿菌的病人會呈現臨床病徵。而低病原性的分枝桿菌感染可以在 HIV 感染個體，老年人與免疫力低下的病人身上觀察到；除此外，某些低病原性感染可以在一些沒有其他感染或發育問題的病人身上觀察到。這些證據推測，臨床上對這些分枝桿菌的易感性與差異性可能來自於人體相關抗分枝桿菌基因的差異性(7)。

卡介苗注射導致感染可能反應出遺傳性免疫缺失(5)。在超過 200 種已知的遺傳性免疫缺失(Primary Immunodeficiency)，部分疾病會呈現出對分枝桿菌的易感性(7)。嚴重合併型免疫缺失(Severe Combined Immunodeficiency, SCID) 病人身上，由於嚴重免疫缺損，病人對大部分的環境微生物均有易感性，由於 BCG 與環境中分枝桿菌為新生兒早期會接觸到的微生物，因此 BCG 與環境中分枝桿菌感染為這些幼童常見的臨床感染症。除此之外，類似的複合性免疫缺失如 Di George syndrome (CDGS)也會導致對 BCG 或是環境分枝桿菌的易感性(5)。此外在 Hyper IgM syndrome (HIGM) (8) 與 Ectodermal dysplasia with Immunodeficiency (EDA-ID) (9)病人中，部分病人也會有 BCG 或是低致病性分枝桿菌感染。

單純因卡介苗注射導致瀰漫性 BCG 感染，而沒有其他特殊免疫缺失或感染的案例第一次在 1951 年被報導(10)。之後，類似的病例也陸陸續續被發現，臨床上，這些病人被定義為因弱致病性分枝桿菌導致嚴重感染，包含 BCG 或是非

結核菌分枝桿菌(Non-Tuberculosis Mycobacterium, NTM)感染，但無其他明顯免疫缺失疾病(5,7,11)。進一步研究發現，這樣對於 NTM 與 BCG 的嚴重感染與 Interferon-gamma Receptor 1 缺失有關(12,13)。因此這類型的疾病被稱作單基因遺傳缺失導致分枝桿菌感染(Mendelian susceptibility to mycobacterial diseases , MSMD)。不令人意外地，MSMD 的病人同樣可能對較致病性的分枝桿菌如結核菌也有易感性。

除了 IFNGR1 缺失之外，另外四個基因缺失也發現會導致 MSMD，包含 IFNGR2 (14,15), IL-12B (16) IL12RB1 (17,18)與 STAT1 (19)。IFNGR1 與 IFNGR2 包含 INF-g受體的 R1 與 R2 兩個蛋白。IL-12B 則表現 IL-12p40，而 p40 為 IL-12 與 IL-23 共同的蛋白。從功能上來解釋，當分枝桿菌感染巨噬細胞(macrophage)或樹突狀細胞(Dendritic cells)時，會引發細胞產生 IL-12 或是 IL-23，而 IL-12 可以藉由細胞上的 IL-12 受體 IL-12RB1 與 B2，刺激 T 細胞或是自然殺手細胞(NK cells)活化，進一步產生 IFN-γ，IFN-γ 可以經由相對應的受體 IFNGR1 與 R2，經由 STAT-1 傳遞抗分枝桿菌的訊號，將巨噬細胞與樹突狀細胞活化，並產生包含 NADPH Oxidase 等基因表現，除去感染的分枝桿菌。因此，MSMD 可能反應出與 IL-12/IFN-γ 這個功能性循環中的基因缺損所導致的分枝桿菌感染(20)。

目前最大的系列報導中，包含有 220 個基因缺失被診斷的 MSMD 病人。其中 5 個基因缺失所佔的比例分別為 IL12RB1 (40%), IFNRG1 (39%), IL12p40 (9%),

Stat-1 (5%)與 IFNRG2 (4%) (20)。其中，IFNGR1 與 IFNGR2 臨床表現與 IL12p40 與 IL-12RB1 缺失略有不同。在 IFNGR1 與 R2 缺失的病人身上，病人大部分在幼年就會呈現 BCG 或是 NTM 的感染，而感染的嚴重程度與突變造成的功能錯失的程度有關(21)，完全性(complete) IFNGR1 與 IFNGR2 基因缺失的致死率相當高，而骨髓移植(Hematopoetic stem cells transplantation, HSCT)是目前唯一的治療方式，但是效果也十分有限(22)。在 IL-12p40 與 IL12RB1 缺失的病人身上，病人雖然同樣有 BCG 與 NTM 感染，但是致死率較低；此外，不同於 IFNGR1 與 R2 缺失病人，IL-12 相關的缺失病人中，約有一半的病人會有沙門氏菌的感染(salmonella)，而且會有復發的現象(23)。STAT-1 是一訊號傳遞分子，除了作用在 IFN-γ 受體下游，同時也會參與 Type I Interferon 的訊號傳遞，因此 STAT-1 基因缺失的病人，根據不同的突變，病人臨床症狀會從單純的分枝桿菌感染(Dupuis et al., 2001)，或是額外會有嚴重型的病毒感染等(24)。

最近的研究發現，在 NEMO 與 CYBB 特殊的基因突變的病人，會呈現出 MSMD 的病症(25,26)。NEMO 為 NF-κB 訊息傳遞中重要的蛋白，此基因位於性染色體 X 上。NEMO 的突變會造成臨床上發育與免疫系統缺失的徵狀，包含女性的 incontinentia pigmenti，或是男性病人 EDA-ID (27,28)。NTM 感染可以在約 30-50%的 EDA-ID 病人身上觀測得到(29)。在研究 MSMD 病人，意外發現 NEMO 突變 E315A 與 R319Q 兩個特殊的突變，相較於其他 NEMO 突變病人，這兩個突變不會造成明顯的發育與免疫系統的缺失。但是進一步研究發現，這兩個位於

特殊功能區 Leucine Zipper 的突變，會干擾單核球或是樹突狀細胞 CD40 的訊息傳遞，導致 IL-12 產生能力下降，而對於分枝桿菌產生易感性。CYBB 突變會導致慢性肉芽腫病 (Chronic Granulomatous Disease, CGD), 臨床上主要的症狀為細菌感染，包含 S aureus 與 Aspergillus fumigatus，分枝桿菌感染較為少見。然而研究發現，兩個 CYBB 特殊的突變 T178P 與 Q231，會導致病人對分枝桿菌有易感性，包含 BCG 與結核菌(25)。相較於一般的 CGD 的突變，會導致 respiratory burst 產生自由基功能的缺失，此兩個特殊的突變幾乎不會造成單核球或是顆粒球(granuolcytes)的 repiratory burst 功能缺失。NEMO 與 CYBB 突變雖然已經發現與其他免疫疾病相關，但由於發現此特殊性的突變，因此這兩個基因也被列入 MSMD 的致病基因中(30)。

(二)台灣地區 BCG 菌株感染

台灣為結核菌高流行性地區，因此從 1965 年起，卡介苗列為新生兒常規注射的疫苗之一。到目前為止，因卡介苗注射引起的 BCG 感染一直都有零星的報導，主要為免疫缺失幼童，成為醫學界與社會關注的議題。台灣地區幼兒分枝桿菌感染診斷，從 2006 年開始疾病管制局加強分枝桿菌分子診斷，根據他們的研究發現，過去台灣地區所使用的 BCG strain Tokyo-172，在台灣地區造成的 BCG 感染，相較於日本過去的紀錄，有較高的感染率，根據疾管局發表的一份研究，在 2005-2008 年間共有 17 例幼兒 BCG 感染的紀錄(31)。另外在 2008-2009 年間，在有通報重症型分枝桿菌感染病例共有 53 例，其中 41 例收到檢驗的檢體中，有

24 例幼兒感染 BCG 的案例(32)。若根據這樣的病例計算，在台灣因 BCG 感染案例接近於每萬名新生兒就會有一例，與國外的經驗相符合(33)。但相較於過去，近年台灣地區 BCG 重症感染的比例似乎有大幅上升的情形，且比較臨床的徵狀，台灣地區的病童的臨床症狀多為重症型的骨髓炎，相較於國外比較高。

在免疫分子診斷方面，林口長庚醫院李文益醫師的研究中，在 271,618 接受卡介苗注射的幼童中，發現 8 名 BCG 感染的幼童。經分子診斷發現有 2 名病人具有 SCID，突變分別為 IL-2 receptor common gamma chain (IL2RG) R226K 與 W74G (34)。在另外一個研究中，同樣在 BCG 感染的病人中，發現 3 名具有 IFNGR1 突變(818del4)的病人與 1 名 IL-12RB1 突變缺失(R211P)的病人(35)。這些結果說明，在台灣地區，卡介苗造成的 BCG 感染，有部分可能是因為宿主因免疫遺傳缺失所造成。另外，在過去幾年間，也僅有少數病人被確診，說明因免疫缺失造成 BCG 感染的角色可能被低估。

(4)本計畫與防疫工作關係

本計畫的目的在為卡介苗感染提出一個可信的宿主遺傳因素。過去幾年媒體對疫苗的負面報導，包含對於流感疫苗與卡介苗的報導，影響人民對疫苗安全與政策的信任。對於卡介苗而言，卡介苗接種還是在目前與可見的未來中，對抗 TB 不可缺乏的疫苗。因此合理解釋卡介苗安全，解釋提出卡介苗感染與疫苗本身安全性無關，且提供分子遺傳診斷，對於這些受卡介苗感染的小孩作為之後治療的依據，等較無關係相關議題對疫苗公衛政策提供重要的依據。

本計畫的主要目的，是研究因包含直接注射卡介苗或接觸其他有卡介苗注射人傳染的 BCG 感染，研究 BCG 的感染與先天性免疫缺失之間的關聯性。本計畫將與疾病管制局合作。以系統性的方式收集台灣地區有 BCG 感染之病童。我們希望本計畫能回答以下幾個問題:

1. 在台灣地區 BCG 感染的幼童中，有多少感染，是肇因於病人遺傳性免疫缺失所造成。
2. 在已知這些與 BCG 與 NTM 感染的遺傳缺失中，台灣地區常見的基因缺失為何?!是否有所謂的突變 Hot Spot 或是 funder effect。
3. 比對遺傳缺失與病人臨床資料，做為診斷分析與流行性醫學的研究。
4. 提供因 BCG 感染的病童，相關的分子診斷結果，提供做臨床治療的參考。

重要性

結核菌感染為在台灣有相當高的流行性率，為我國公共衛生上的主要威脅。目前為止，卡介苗還是唯一可以用來對抗結核菌感染的疫苗。卡介苗的保護力有限，加上其為活疫苗，因此其效用與潛在的危險性一直是公衛上討論的課題。另外，目前研發中的抗結核菌疫苗，仍然主要是根據卡介苗修改的減毒疫苗(36)，因此卡介苗造成的感染問題，很可能這些疫苗也會有類似問題。自近年來，疫苗安全一直是國內輿論與公共衛生界主要爭論的議題，因此針對卡介苗的安全性研究刻不容緩。

疫苗造成的感染可能來自於疫苗株本身的致病性，製備過程的汙染或是病人本身的易感性(包含先天性與後天性的問題)。台灣地區因 BCG 感染的機率與國外各國類似，且不具有流行性或是群聚性，說明前兩種造成的疫苗感染可能無法解釋台灣地區所發生的 BCG 感染。因此我們相信，免疫缺失所造成的感染可能是台灣地區 BCG 感染的主要因素，也與過去研究結果相符合(Lee et al., 2009b)。我們希望藉有系統地研究 BCG 感染與免疫缺失間的關係。這樣的研究結果，可以馬上應用於病人的臨床治療，過去診斷過一例 CYBB 基因缺失造成 NTM 感染的男童，因分子診斷確認後，CGD 長期預後不良，因此病人接受異體臍帶血幹細胞移植，目前已經康復。希望藉由分子診斷，能讓更多的病人有機會確診與接受更好的治療。除了臨床意義外，本研究計畫結果做為未來卡介苗接種計畫規範時的依據與參考。並希望藉由此研究，釐清卡介苗感染的問題，重建民眾對卡介苗安全的信心。

另外希望藉由大量有系統的分析，了解台灣地區 MSMD 基因缺失的類型，包含相關基因突變的病人數，分布區域，與其突變的位置，與其特殊性，希望對了解這些基因的功能與病人臨床徵狀上做連結。研究是否在台灣地區有族群的特異性，或是特殊的突變。

最後，本計畫將建立一個 BCG 感染的資料庫，作為研究 MSMD 的基礎。目前研究指出，已知的基因缺失可以解釋約一半的重症 BCG 或 NTM 的幼兒感

染；然而，還有超過一半的病人其原因不明，藉由本計畫，區分出無法以已知基因缺失解釋的 BCG 感染病人，計畫在未來的研究中，能找出新的基因缺失，了解更多人類抗分枝桿菌的分子機制。

研究目標

1. 有系統性收集台灣地區因卡介苗注射導致 BCG 感染與嚴重 TB 感染的幼童。
2. 以臨床檢測與功能性檢測方式分析可能的免疫缺失
3. 以基因定序的方式找出可能的遺傳疾病
4. 整合研判個案之臨床功能資料與基因定序分析結果，就未來個案收案數與關聯性分析樣本需求進行評估

研究方法

1. 有系統性收集台灣地區因卡介苗注射導致 BCG 感染的幼童。

本計畫主要將與國內大型醫學中心合作，並根據疾管局分析發現的卡介苗感染幼童病例為標準，以有系統的方式收案因卡介苗注射導致 BCG 感染，或是因社群傳播導致 BCG 感染的幼童。收案的條件如下:

納入標準

I. 因卡介苗菌株 BCG 造成重症感染的幼童(除了注射處外的感染，包含骨髓炎，瀰漫性感染等)，包含直接注射卡介苗或接觸其他有卡介苗注射的人造成的傳染。
II. 願意簽署書面受試者同意書

排除條件

I. 患有愛滋病(HIV 感染)

II. 有長期服用免疫抑制藥物

依據病童的年紀收集 1.5-5ml 的血液樣本(一歲以下收集 1-1.5ml，一至兩歲收集 1.5-3ml，兩歲以上收集 3-5ml)，並以問卷詢問基本的家庭背景（如生活環境，祖籍，家中人數，家中特殊病史），以及相關的病史。採集血液次數。以不超過三次為原則，且每次間隔一個月以上。在病人有可疑的遺傳缺陷的狀況下，我們將採集父母的血液樣本，作為 DNA 分析時的佐證資料。

另外，MSMD 病人可能有 disseminated NTM 或是 TB 感染，為了瞭解 MSMD 是否也能解釋這樣的分枝桿菌感染，我們也將同時收案有非肺部嚴重感染的 TB 幼童，同樣做免疫遺傳分析。嚴重 TB 感染定義為非肺部(extra pulmonary)TB 感染，包含骨髓炎，腦炎...與瀰漫性 TB 感染等。納入條件為願意簽署受試者同意書，且無 HIV 感染或長期服用免疫抑制藥物之兒童。

2. 設立功能性檢測

根據已知與卡介苗感染相關的單基因缺失疾病，我們將設立以下實驗流程

I. IL-12/IFN-γ 功能檢測

根據過去建立之系統(Feinberg et al., 2004)，在 24 孔盤中，將 Heparinized 血液檢體以 1:1 方式稀釋在含有 1% P/S (100U/ml penicillin +100μg/ml streptomycin) 的

RPMI1640 中，加入各種細胞激素 BCG (50μl), BCG+IL12 (20ng/ml), BCG+IFN-γ (250ng/ml) 刺激細胞活化反應。在 37 ºC, 5% CO2 培養箱中反應 48 小時後，吸取上層血清以轉速 12000rpm 離心 10 分鐘後，將上清液移入新的 1.5ml tube 管中，存放在 -80 ºC 冰箱。隨後以酵素免疫分析法 (ELISA) 偵測血清中 IL-12 和 IFN-γ 的細胞激素產生量。

II. 自由基產生能力測試

Heparinized 血液檢體取 50ul 放入分析管中，加入 1x Red lysis buffer 1ml 震盪 10~15 秒鐘，室溫下放置 10 分鐘，以轉速 1700rpm 離心 5 分鐘，將上清液倒去後再以 1xPBS 2ml 清洗，以 1700rpm 離心 5 分鐘後去除上清液，加入 PMA (100nM) 1μl 震盪 3 秒鐘，放入 37 ºC 培養箱中刺激 20 分鐘，取出再加入 Dihydrorhodamine 123 (DHR123, 3μl/ml) 1μl 染色 15 分鐘，最後加入 500μl 1x PBS 放置 4 ºC 保存避光。檢體再以流式細胞儀分析其產生自由基的能力。

III. NF-kappaB 功能缺失檢視

參考過去研究(9)，在 24 孔盤中，將 Heparinized 血液檢體以 1:1 方式稀釋在含有 1% P/S (100U/ml penicillin +100μg/ml streptomycin) 的 RPMI1640 中，加入細胞激素 LPS (100ng/ml), TNF (20ng/ml) 刺激細胞活化反應。在 37 ºC, 5% CO2 培養箱中反應 48 小時後，吸取上層血清以轉速 12000rpm 離心 10 分鐘後，將上清液移入新的 1.5ml tube 管中，存放在 -80 ºC 冰箱。隨後以酵素免疫分析法 (ELISA) 偵測血清中 TNF-a和

IL-10 的細胞激素產生量。

3. 結果

病人收案狀況

本計畫共收案 7 位 BCG 確診感染個案與 1 位 TB 重症感染病人。病人年紀從 3 個月到 3 歲 10 個月。主要感染為骨髓炎(osteromyelitis)與淋巴結感染；除了病人(BCG8)外，病人主要感染已深部組織為重症定義，但是並無瀰漫性感染的症狀。BCG8 號病人呈現明顯注射處與下肢皮膚組織膿腫，並伴隨淋巴結症狀，MRI 掃描顯示病人在骨髓炎的現象與敗血性關節炎，因多處組織感染，病人顯示出瀰漫性 BCG 感染的問題。

功能檢查並無病人有 IL-12/IFN-γ，NEMO 與 TLR 功能缺失

對病人的血液檢體做功能性檢測。病人對於 IFN-γ 與 IL-12 刺激後，相對應的細胞激素製造均反應正常，說明並無病人有 IL-12/IFN-γ 功能軸上的基因缺失問題。另外，過去研究也發現 NEMO 基因缺失的病人也可能有類似的 BCG 感染問題，因此我們也測試 TNF 刺激後 IL-10 生成狀況，並無異常發生。我們另外利用 CD62L shedding assay，以 TLR4 的配體 LPS 與 TLR7/8 配體 R848 刺激，觀察病人多核球上 CD62L 因活化消失的現象，檢測個體中均反應正常，說明無 TLR 基因缺失的問題。

ISG15 定序結果並無異常

ISG15 基因缺失最近發現與 NTM 感染有重要的關係，由於此基因缺失無法以功能性檢測發現，因此我們直接在所有病人上對此基因做直接定序。定序結果除了找到

部分核甘酸多型性外(Val, A294G)，並無發現相關的基因缺失。

BCG8 號病人對於自由基(Free Radical)製造有缺失

利用 PMA 刺激後，已染劑 DHR 觀察多核球上的自由基產生的狀況。病人刺激後反應均正常，除了 BCG8 有刺激後沒有明顯反應(見附圖)。

BCG8 號帶有 CYBB 基因突變

BCG8 號病人為男性，從六個月起因 BCG 注射處感染住院，血液常規檢查與臨床症狀排除 SCID 的可能性；病人來自於非近親婚姻的小孩，有兩個年長個哥哥因為不明原因的感染引發敗血症，在嬰兒期死亡。因家族史病例與受到感染的病例皆為男生，因此高度懷疑有性染色體連結(X chromosmoe linked)的基因缺失。在 X chromosome 上 NEMO 與 CYBB 兩個基因都被報導與人類先天免疫缺失與細菌感染有關。以病人 cDNA 定序排除病人有 NEMO 基因缺失的可能性，CYBB 為產生自由基的蛋白，此另外以 cDNA 定序發現病人在 CYBB 上有一個突變 665A>G (H222R)，此突變已在文獻中被報導與 CGD 有關。另外，利用母親的檢體中發現，母親帶有此突變的異型合子。

病人在 19 個月大時接受臍帶血幹細胞移植，移植後病人骨髓重建成功，多核球的自由基功能製造功能正常，感染問題也解決，相關結果已發表在相關學術期刊上(請見附件)。

4. 討論

卡介苗菌株所造成的重症感染為卡介苗使用上的一個問題。我們希望分析台灣地區因注射卡介苗感染的病童的，了解其感染是否為宿主免疫基因缺失所造成。在我們分析的 7 位卡介苗感染的病童中，有一位病童的確有 CYBB 基因缺失，其卡介苗注射所引發的瀰漫性感染為明顯的宿主免疫缺失造成。因此卡介苗疫苗的嚴重副作用引發的感染症中，宿主因素是需要考慮的一個原因。

卡介苗為減毒性的活體疫苗，對於免疫不全的宿主來說會有明顯的副作用，像是嚴重合併型免疫缺失病人中，卡介苗疫苗會導致致命性的感染。然而，卡介苗在部分看似正常的幼童身上，也會有重症感染的問題，此一現象一直不清楚原因，疫苗菌株突變，生產汙染或是菌株安全性一直被高度懷疑。但是在過去近 15 年間，IL-12/IFN-γ 相關基因缺失發現後，了解部分卡介苗重症感染的病人，是因為新型免疫遺傳缺失所造成，而這些基因是無法被傳統的臨床檢驗發現。本計畫也是延續此一概念，希望能藉由分析台灣地區病童的遺傳相關缺失，釐清此一因素。IL-12/IFN-γ 相關基因缺失為幼兒 NTM 或是卡介苗感染最主要原因，大約超過 7 成以上已知被確診有基因缺失的相關感染都是由此相關基因缺失造成，包含 IL-12RB1, IL-12B, IL12RB2, IFNGR1, IFNGR2, STAT1 等。過去在台灣地區，IFNGR1 與 IL-12RB1 基因缺失曾經被報導(35)，但是在本研究中，我們並未找到這樣的病人。可能的原因是本計畫收案量低於預期值，可能使得統計上有偏差性，另一個可能是為本收案為 BCG 感染，病人症狀較遺傳缺陷所造成的感染(主

要是瀰漫性感染)要來的輕微，所以沒有看到類似的病例。結合以上的可能，遺傳性基因缺失在瀰漫性卡介苗染感中可能有比較重的角色。因此在觀察疫苗安全與宿主因素時，臨床上的病人的嚴重性的鑑定與分類是需要考量的因素之一，需考量台灣地區卡介苗感染較國外常見，是否與台灣地區對卡介苗感染回報的條件較為寬鬆所導致的。

CGD 是常見的幼兒遺傳免疫缺失，無法以臨床血液常規檢發現，相關的功能性檢查已經非常成熟，然而不是所有的醫院都具有這檢查的能力，目前為止此項檢查並無列入台灣地區新生兒篩檢項目。CGD 中以 CYBB 突變最為常見，約佔所有 CGD 病人的 2/3。然而 CGD 的臨床症狀中，主要以 S aureus 或是其他細菌性感染，黴菌感染，像是 Aspergellus 屬的黴菌感染也被報導。由於遺傳性免疫缺失惟一般臨床醫師較少見到的疾病，對此一疾病的認識不足；尤其卡介苗感染並非 CGD 的標準型症狀，因此在臨床醫師面對卡介苗感染時常常會忽略。CGD 的治療方式，目前骨髓移植及臍帶血移植的效果都相當好(37)，相關的基因治療也有不錯的成果(38)，協助病人的早期確診，在醫療上對病人是非常重要的。

雖然卡介苗在過去大規模使用的經驗上，說明這疫苗是相對安全。但是這疫苗的保護性有限，加上在少數病人身上的嚴重甚至致命性的感染的副作用，卡介苗目前使用上的疑慮不小。遺傳性免疫缺失疾病比過去想像的常見，這些病人若接種卡介苗會有極嚴重的問題，最近研究顯示嚴重合併型免疫缺失盛行率約為 1/19000

(39)，這樣的病人若接受卡介苗注射將會有明顯的反應。因此目前各國，包括台灣地區，都在推行嚴重合併型免疫缺失的新生兒篩檢(39)，以及延後施打卡介苗的時間，這樣對於此類病童的存活率有重要的意義。然而，還有許多免疫基因缺失與卡介苗重症感染有關，且無法以簡單的方式做篩檢，因此若相關基因缺陷病童接受卡介苗注射，對病童會有嚴重的副作用甚至死亡，對社會來說也會對疫苗安全有極大的疑慮。再考慮卡介苗的保護力有限下，是否強制性全國施打卡介苗為一個值得討論的問題。

另外，雖然我們在大部分的病人身上無法找到基因缺失，並不代表我們可以排除宿主因素，在國外研究也指出，還有一大部分瀰漫性分枝桿菌重症感染的病童，無法找出基因缺失解釋其易感性。近年持續有需多基因陸陸續續被報導與幼童分支桿菌重症有關，像是 NEMO 與 CYBB 等，因此可能還有許多未知的易感基因，未列入本計畫的分析。另外，遺傳疾病有高度的地區與種族差異，具例來說 IL-12B 基因缺失在印度與沙烏地阿拉伯半島較為常見，可能與一個早期的 founder 突變有關(40)，過去的研究均較少以台灣地區或是華人為主要的研究對象，很可能台灣地區有特殊的易感性基因未被發現。近年遺傳工具的進步，包括像是全基因定序的技術，加速尋找這些遺傳性疾病的致病基因，最近利用這技術就找到 ISG15 與分枝桿菌感染的關聯性，另外也發現 AP4E1 可能與卡介苗易感有關(41)。因此我們也利用全基因外顯子定序尋找我們病人可能的新型基因缺失，由於該技術耗時較久，並需要長時間分析，因此無法在本計畫時限內完成呈現。該分析正在進

行中，相關結果也將會對我們了解卡介苗感染的宿主因素有更深入的認識。

5. 建議

- 本計畫在收案上遇到相當的困難，因此未收足相關病例，造成結論較為薄弱。原因在於目前 IRB 相關要求與通報上的問題。卡介苗注射副作用案例，常會藉由疾管局協助菌株鑑定中發現，另外部分案例會因副作用申請藥害救濟時回報相關單位。然而因個人隱私保護，我們無法得到相關的資料，因此在病例收集上較為困難，僅能從部分合作單位收集相關病例。另外，病人相當分散在各醫院，在發現個案後，需要從送該院的 IRB 審查，以短期計劃來說時間上幾乎沒有辦法。建議相關研究，是否可以疾管局或是國家機構的 IRB 來協助收案研究；或是藉由疾管局或是相關單位，在發現個案後，主動告知病患與所負責照顧之醫師，相關國內有能量研究此一問題的實驗室與單位，要求他們主動聯絡尋求協助。以減少實際上的問題，真的達到協助病人與醫師的目的。

- 在過去研究上發現，瀰漫性卡介苗感染中，與宿主基因缺失的關聯性極高。本計畫中唯一找到基因缺失的病人，也屬於瀰漫性感染的例子，說明宿主單基因免疫缺失可能在臨床上的症狀，會是屬於瀰漫性感染的問題。在台灣地區卡介苗副作用觀察到的，多數的病人數為注射處附近的淋巴感染，分類上屬於較輕症。建議將 BCG 感染的病童，要求做較完整的檢驗，像是 MRI 等全身性檢查，將病人依據感染的位置與嚴重度分類，對於重症病人後續相關治療與處理極為重要。

- CGD 為常見的免疫缺失，目前臨床教學上，並不強調此疾病與卡介苗或是分支桿菌感染的相關性。然而近期的研究與本計畫成果，說明 CGD 為卡介苗或是分枝桿菌易感基因之一。建議若病人有嚴重的卡介苗感染，雖然無其他化膿性細菌感染或是黴菌感染時，還是須將 CGD 列入可能的考慮的方向。

- 在卡介苗保護力有限，而在免疫缺失的小兒上會有無可預測的嚴重副作用狀況下，是否全國性強制施打卡介苗，為一個值得討論的議題。

- 基因性疾病有種族或是地區性的特異性，台灣地區較高的卡介苗感染因素，除了疫苗本身的菌株還有製造狀況外，宿主因素，包括可能是新型未知的免疫基因缺失所造成，仍不能排除。尤其相關的研究中，以華人或是台灣人族群的研究相當的少，無法排除此一可能性。也可能解釋為何我們僅在 8 個病人中找到一個宿主因素缺陷。未來相關研究應往這方向進行。目前部分病人以全外顯子定序持續分析中，希望可以有部分結果。

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7. 圖表

Table I-1. The Functional Test and ISG15 gDNA Sequencing of BCG01 patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG01	Female	2yr-4mon	-	-	Normal	Homozygous	Homozygous

Figure 3-1. ELISA IFN-r and IL-12p40 Set of Control VS. BCG01

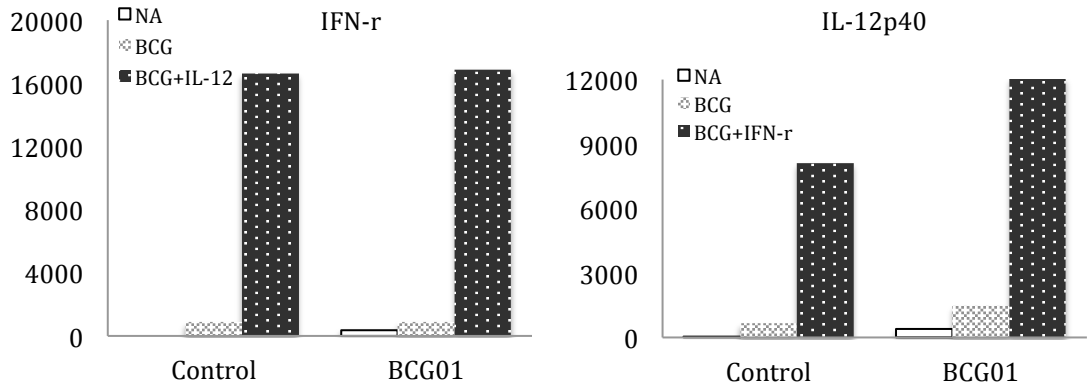
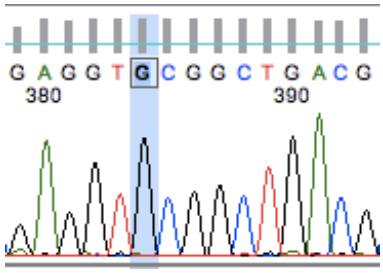


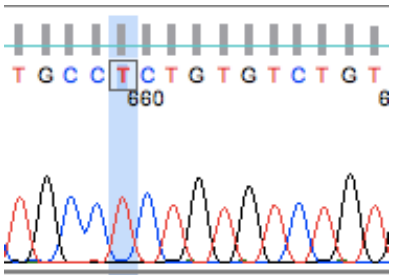
Figure 4-1. ISG15 Sequencing of BCG01

SNP1: Val98=A>G
Forward



Homozygous

SNP2: rs2799070C>T, 3'near gene
Forward



Homozygous

Table I-2. The Functional Test and ISG15 gDNA Sequencing of BCG02 patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG02	Male	1yr-3mon	-	Normal	Normal	Homozygous	Homozygous

Figure 1-2. The CD62L Shedding of Control VS. BCG02

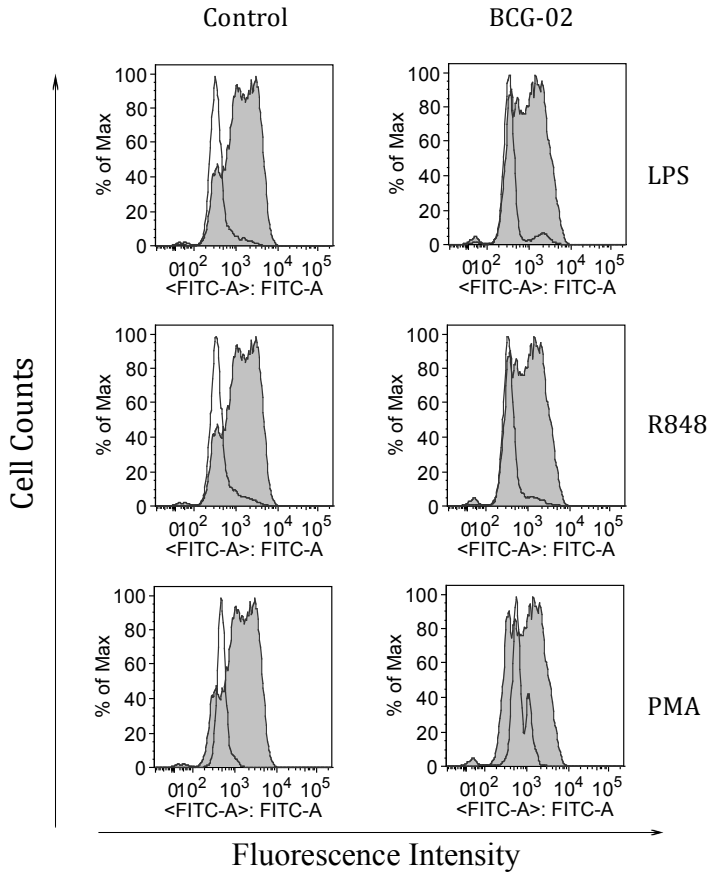


Figure 3-2. ELISA IFN-r and IL-12p40 Set of Control VS. BCG02

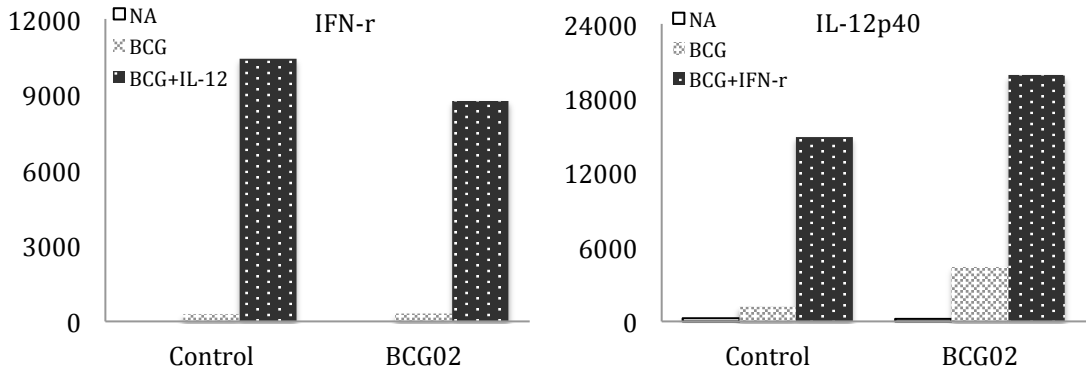
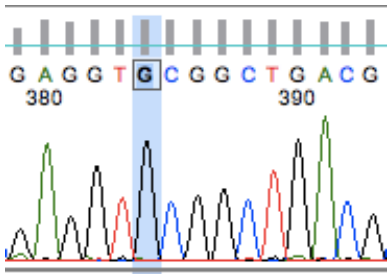


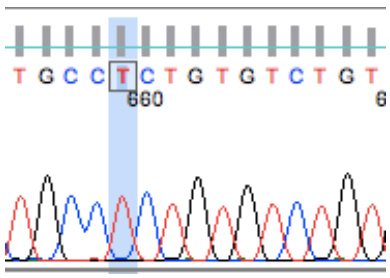
Figure 4-2. ISG15 Sequencing of BCG02

SNP1: Val98=A>G
Forward



Homozygous

SNP2: rs2799070C>T, 3'near gene
Forward



Homozygous

Table I-3. The Functional Test and ISG15 gDNA Sequencing of BCG03 patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG03	Male	3yr-10mon	Normal	Normal	IFN-r, IL-12p40(low)	Homozygous	Homozygous

Figure 1-3. The CD62L Shedding of Control VS. BCG03

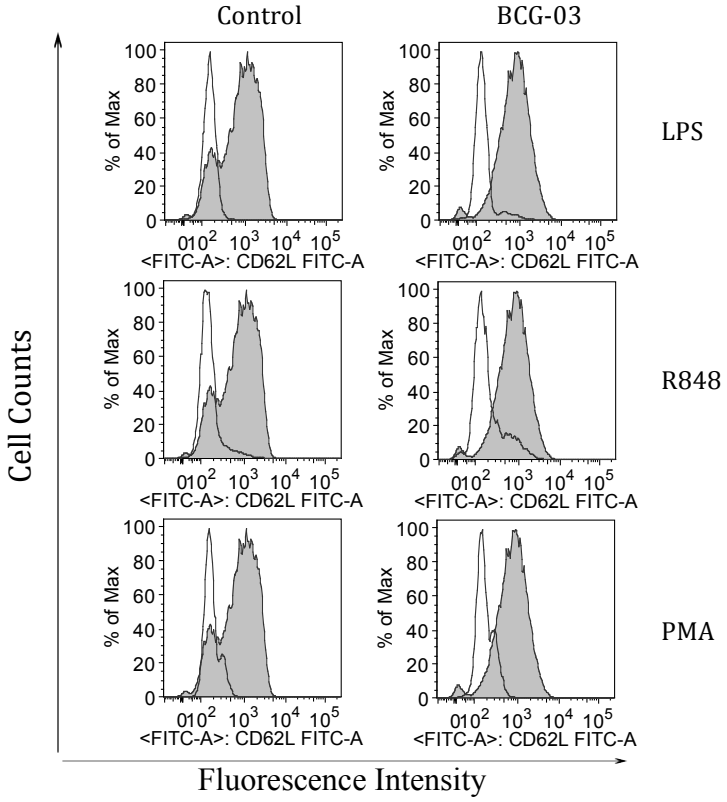


Figure 2-3. The DHR of Control VS. BCG03

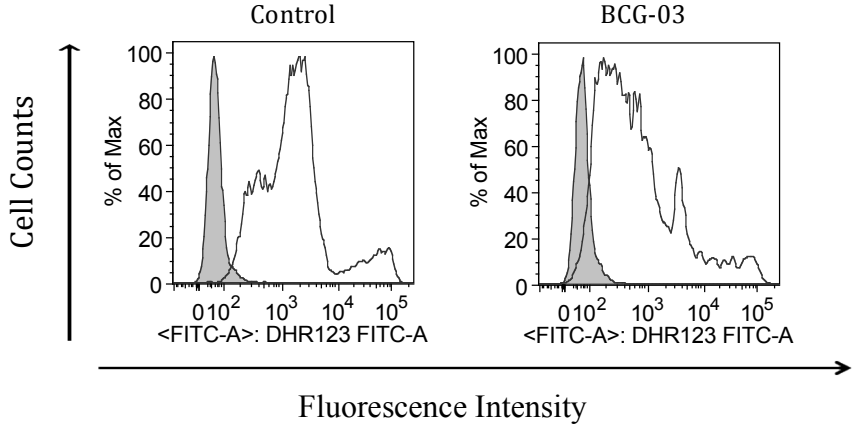


Figure 3-3. ELISA IFN-r and IL-12p40 Set of Control VS. BCG03

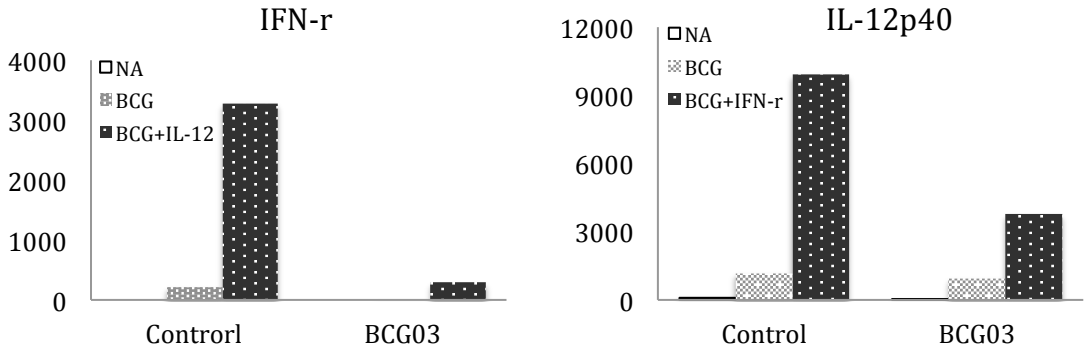
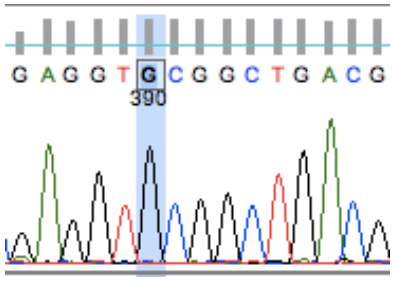


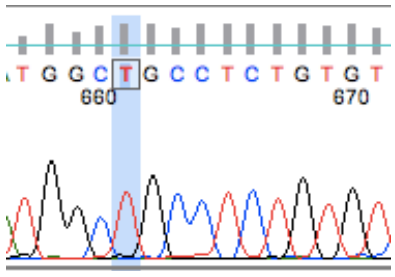
Figure 4-3. ISG15 Sequencing of BCG03

SNP1: Val98=A>G
Forward



Homozygous

SNP2: rs2799070C>T, 3'near gene
Forward



Homozygous

Table I-4. The Functional Test and ISG15 gDNA Sequencing of BCG04 patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG04	Female	1yr-2mon	Normal	Normal	IFN-r, IL-12p40	Homozygous	Homozygous

Figure 1-4. The CD62L Shedding of Control VS. BCG04

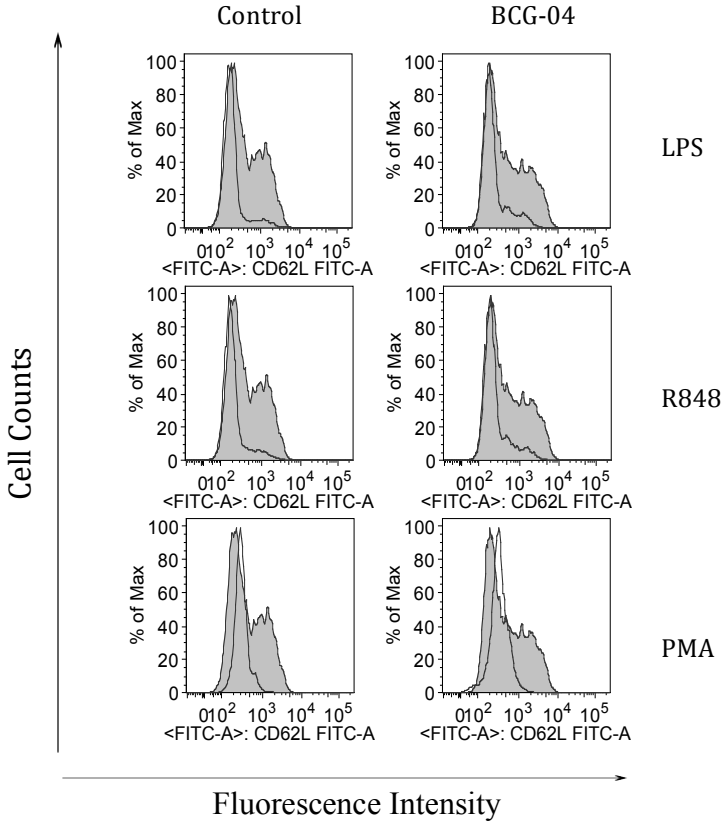


Figure 2-4. The DHR of Control VS. BCG04

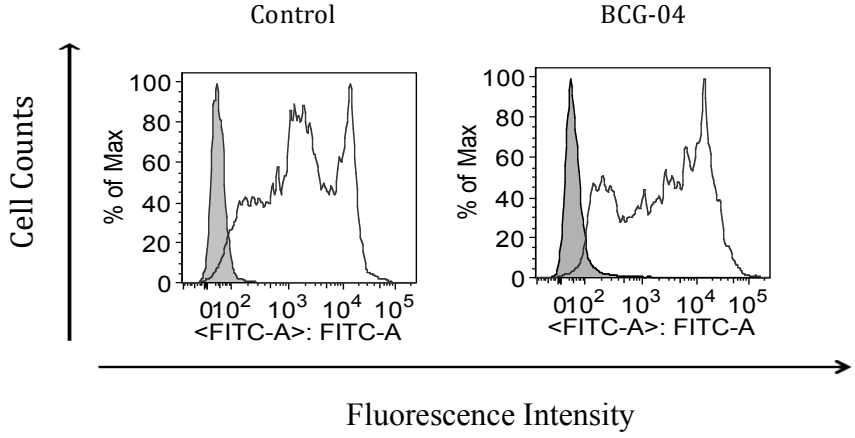


Figure 3-4. ELISA IFN-r and IL-12p40 Set of Control VS. BCG04

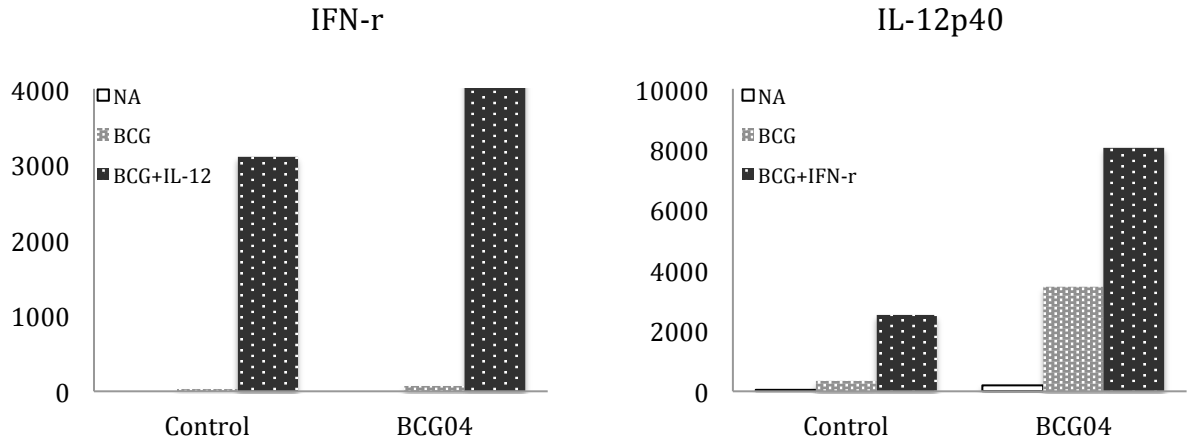
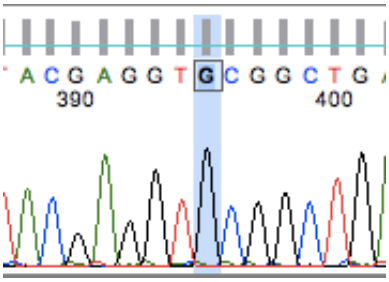


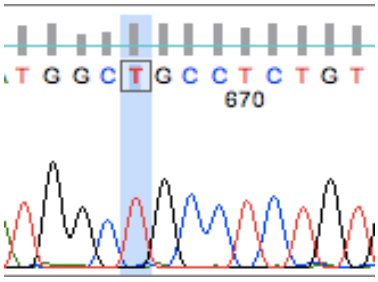
Figure 4-4. ISG15 Sequencing of BCG04

SNP1: Val98=A>G
Forward



Homozygous

SNP2: rs2799070C>T, 3'near gene
Forward



Homozygous

Table I-5. The Functional Test and ISG15 gDNA Sequencing of BCG05(TB) patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG05	Female	3mon	Normal	Normal	IFN-r, IL-12p40	-	-

Figure 1-5. The CD62L Shedding of Control VS. BCG05

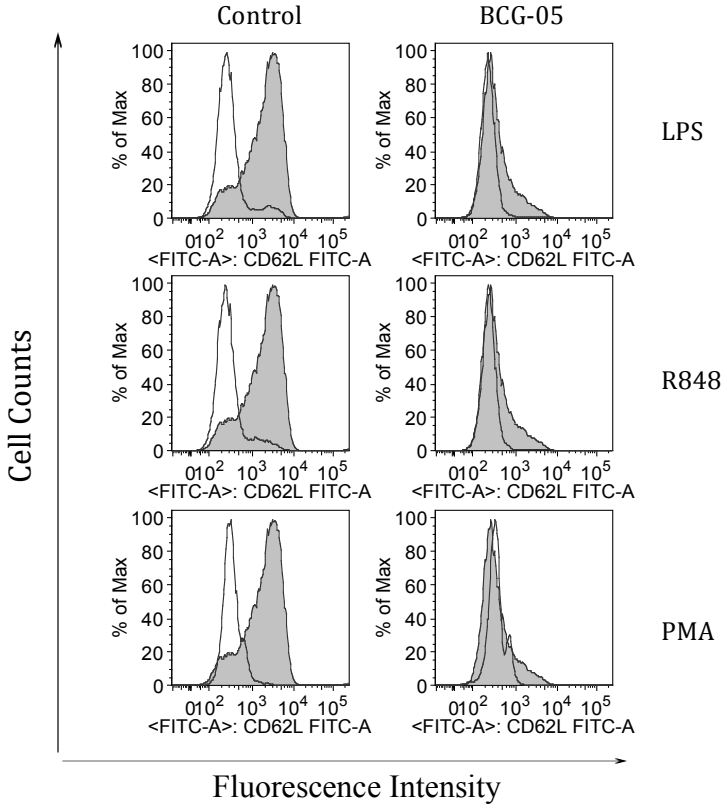


Figure 2-5. The DHR of Control VS. BCG05

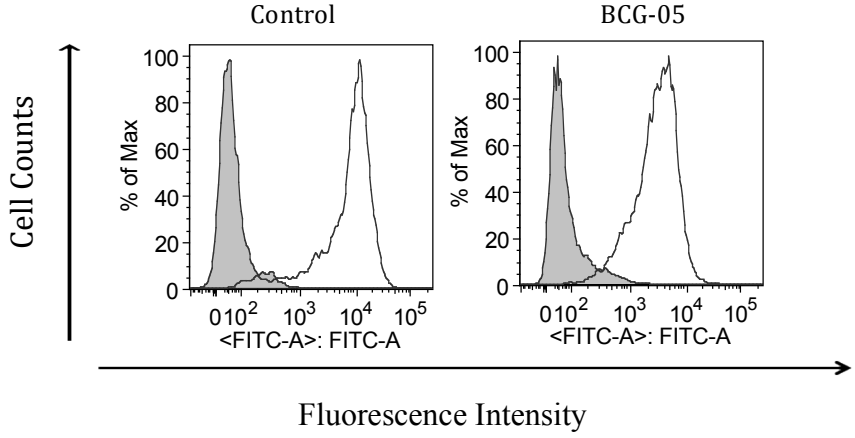


Figure 3-5. ELISA IFN-r and IL-12p40 Set of Control VS. BCG05

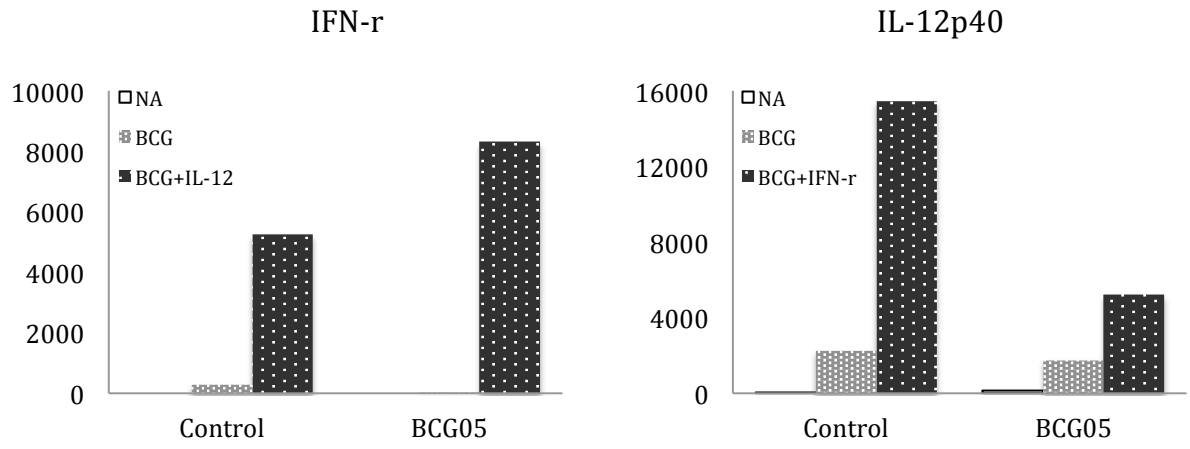


Table I-6. The Functional Test and ISG15 gDNA Sequencing of BCG06 patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism	
						Val98=A>G	rs2799070C>T, 3'near gene
BCG06	Male	1yr-6mon	Normal	Normal	IFN-r(low), IL-12p40	Homozygous	Homozygous

Figure 1-6. The CD62L Shedding of Control VS. BCG06-1

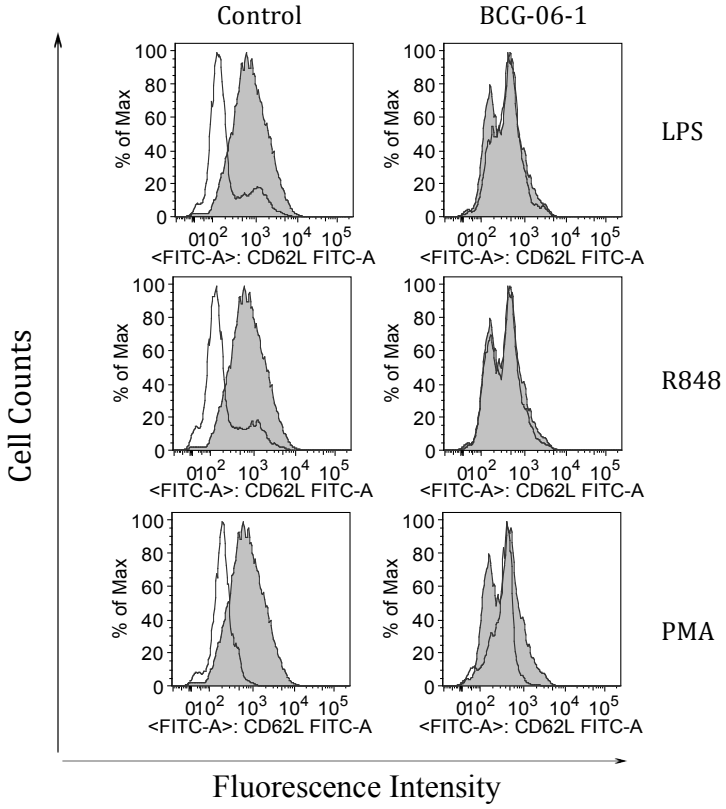


Figure 1-6. The CD62L Shedding of Control VS. BCG06-2

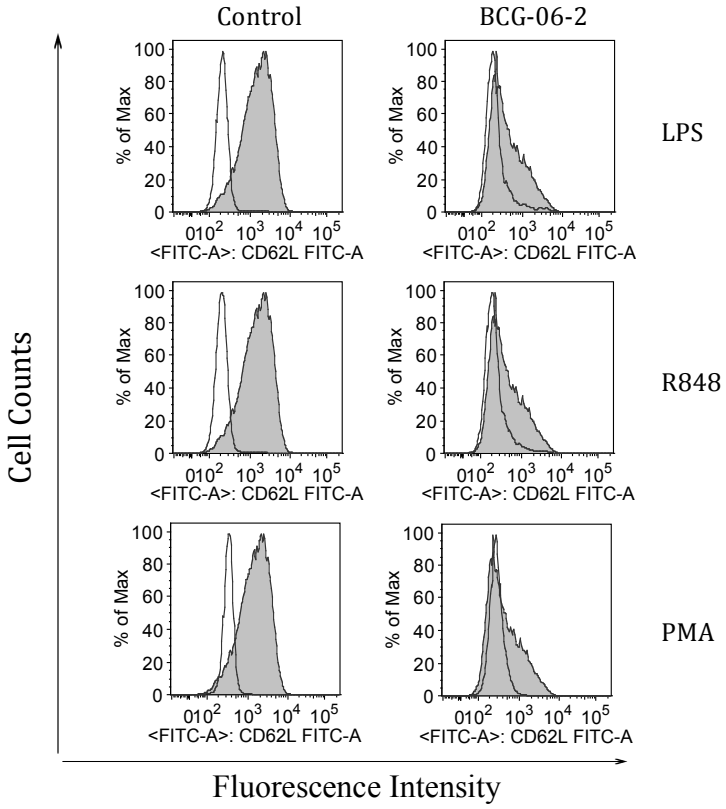


Figure 2-6. The DHR of Control VS. BCG06

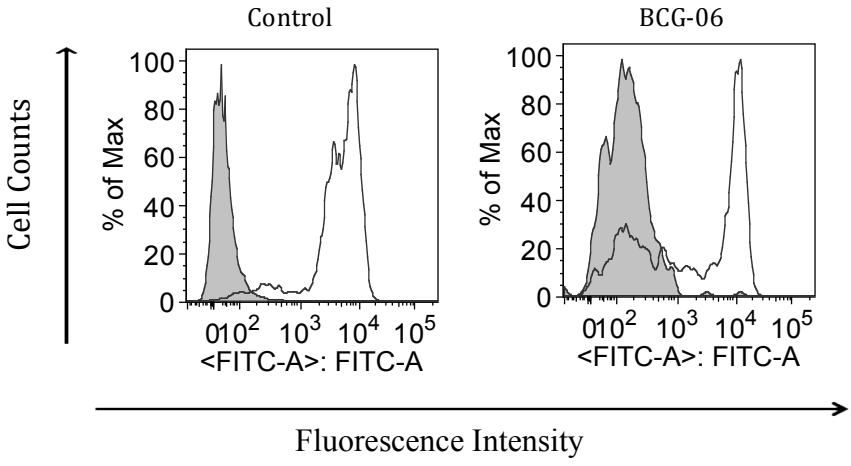


Figure 3-6. ELISA IFN-r and IL-12p40 Set of Control VS. BCG06

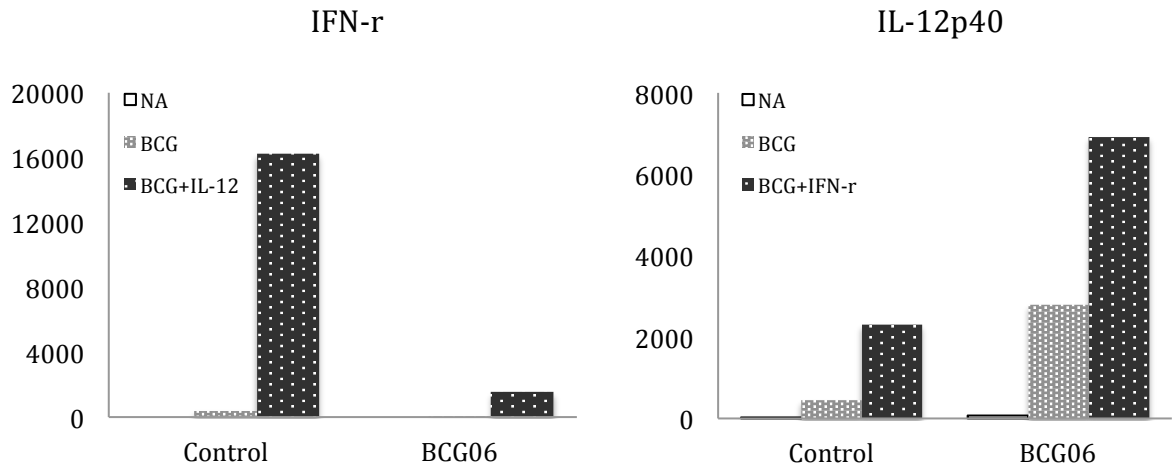
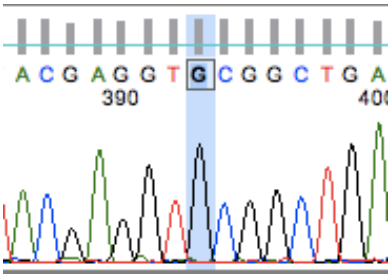


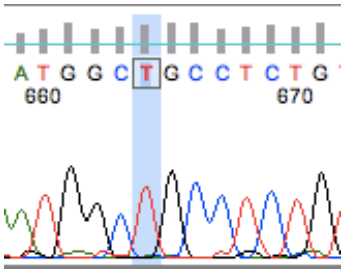
Figure 4-6. ISG15 Sequencing of BCG06

SNP1: Val98=A>G
Forward



Homozygous

SNP2: rs2799070C>T, 3'near gene
Forward



Homozygous

Table I-7. The Functional Test of BCG07(NTM) patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	ISG15 Polymorphism Val98=A>G	ISG15 Polymorphism rs2799070C>T, 3'near gene
BCG07	Male	5mon	-	Normal	IFN-r, IL-12p40	-	-

Figure 1-7. The CD62L Shedding of Control VS. BCG07

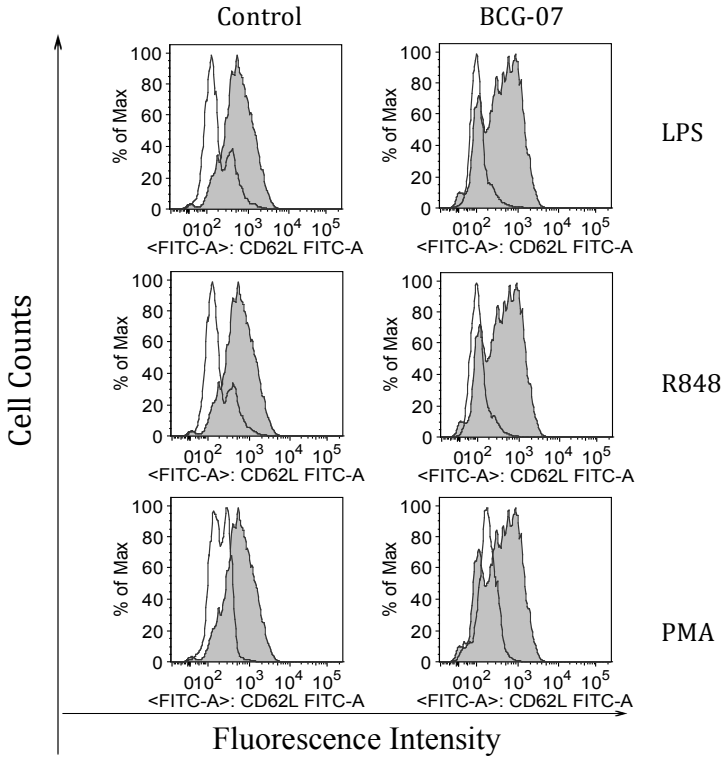


Figure 3-7. ELISA IFN-r and IL-12p40 Set of Control VS. BCG07

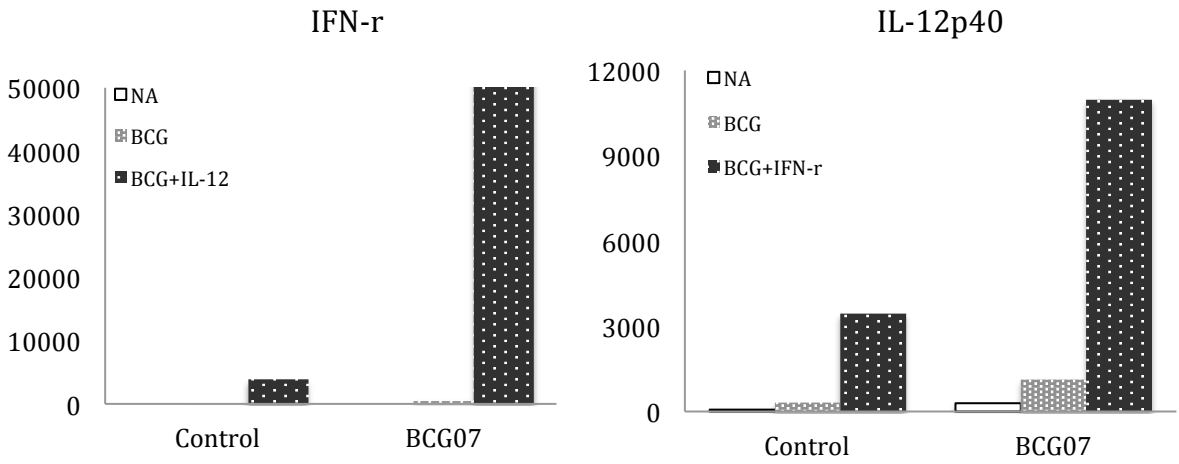


Table I-8. The Functional Test and CYBB gDNA Sequencing of BCG08(CGD) patient

Patient	Sex	Age	DHR	CD62L shedding	WB stimulation	CYBB Deficiency Exon7 665A>G His222Arg
BCG08	Male	10mon	No Function	No Function	IFN-r(low), IL-6	Homozygous

Figure 1-8. The CD62L Shedding of Control VS. BCG08

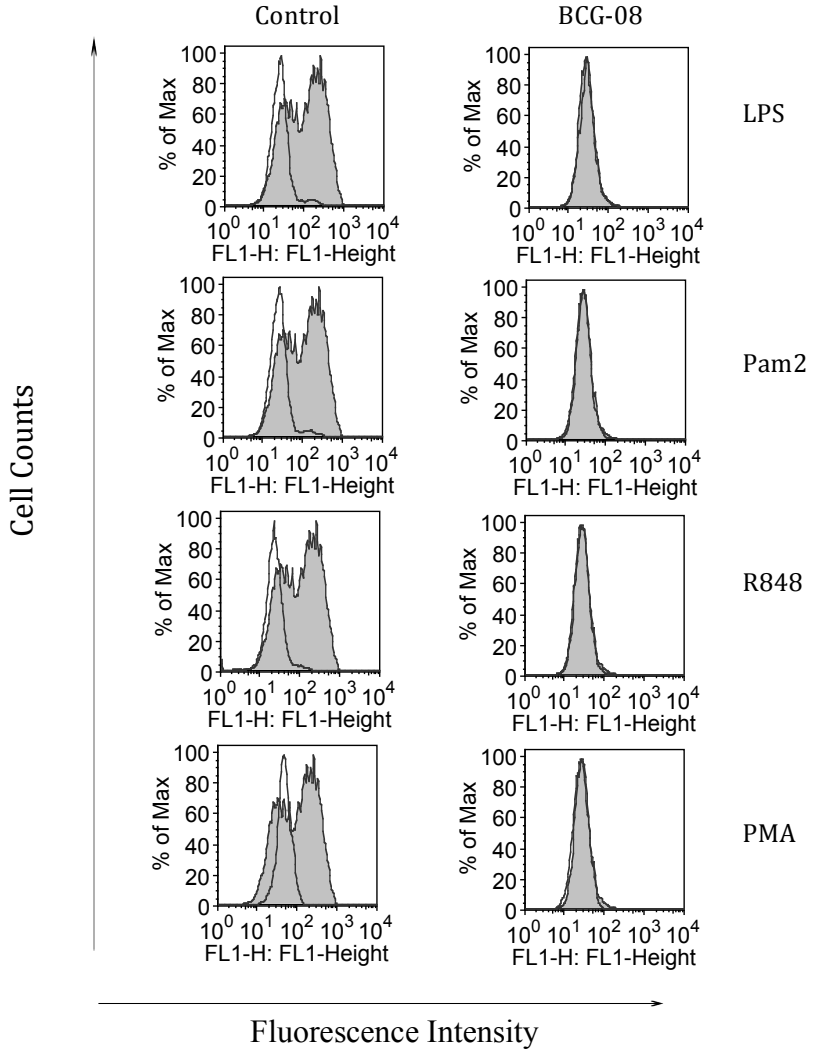


Figure 2-8. The DHR of Control VS. BCG08

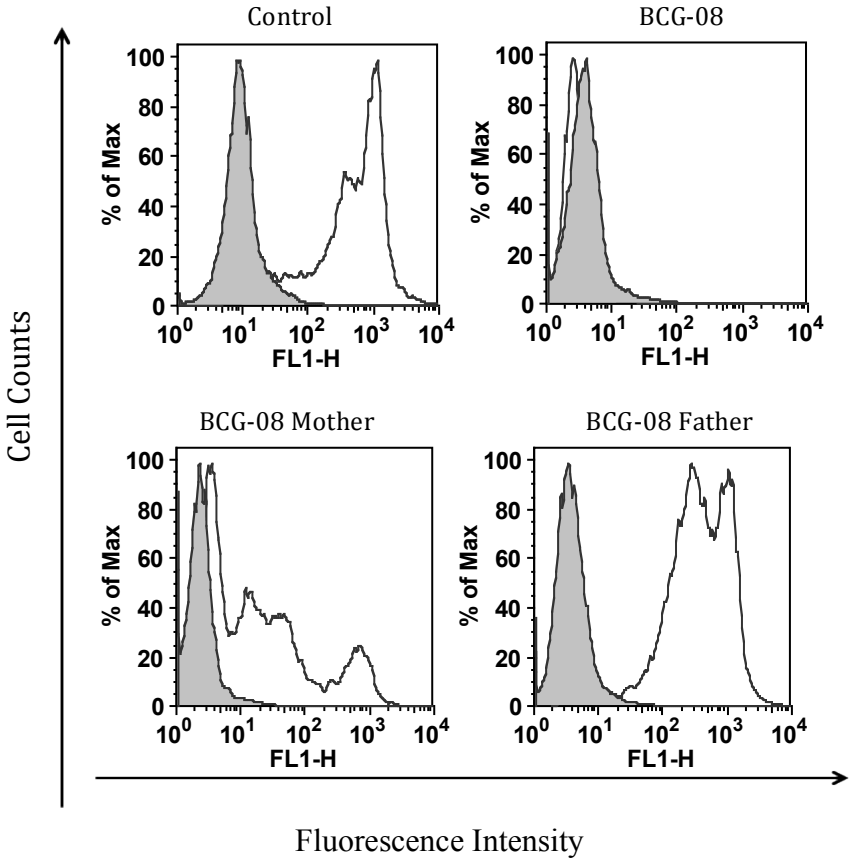


Figure 3-8. ELISA IFN-r and IL-6 Set of Control VS. BCG08

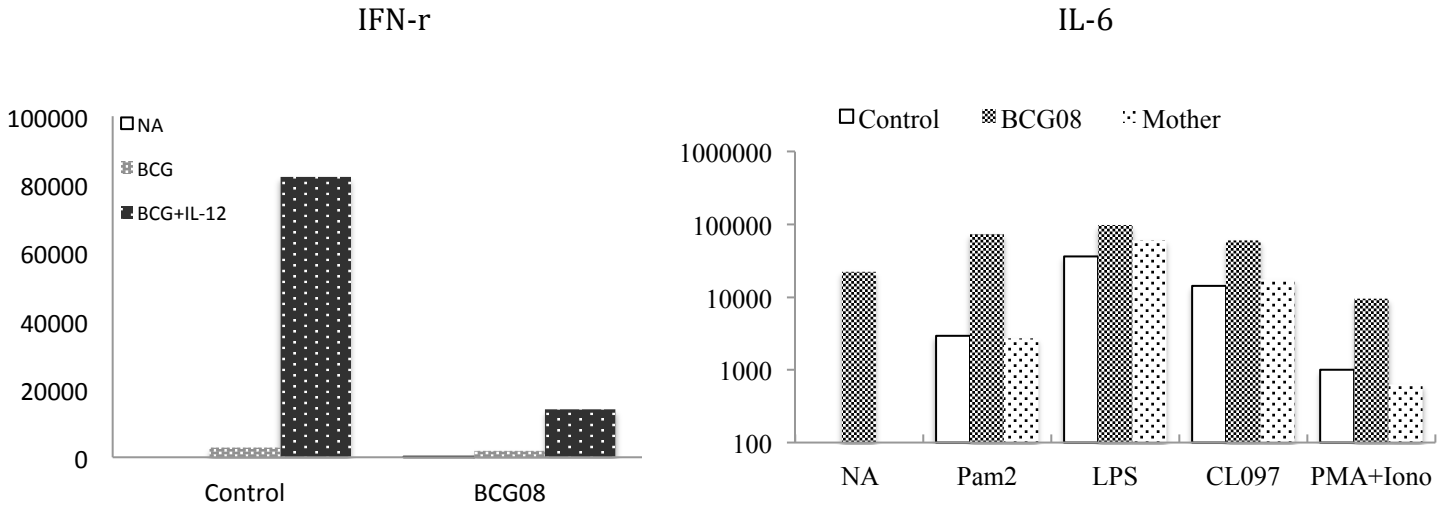
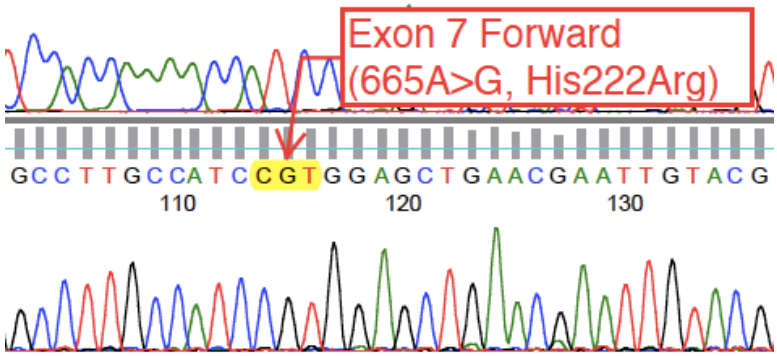


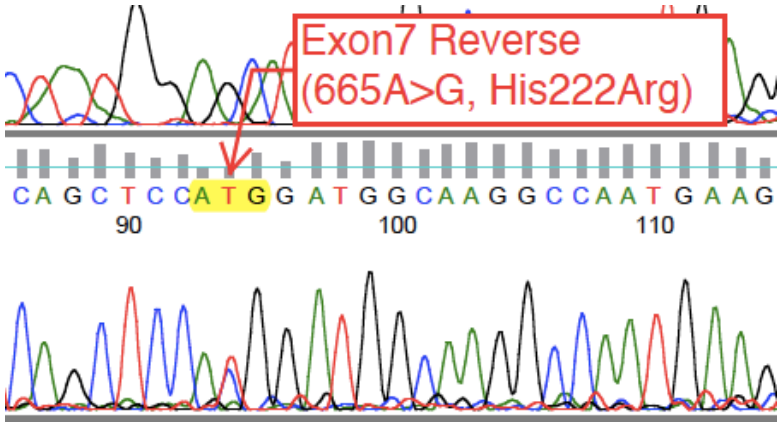
Figure 4-8. CYBB Sequencing of BCG08

BCG08



Homozygous

BCG08 Mother



Heterozygous

Table II: Primer sets for the sequence

ISG15 gDNA

NCBI Reference Sequence: NC_000001.10		
Forward	5’- CGGGATGTAGAGGACAGACA -3’	825 b.p
Reverse	5’- ACCCTTATCCCTTCACTTGG -3’	

CYBB gDNA

NCBI Reference Sequence: NC_000023.10		
Forward	5’-CTGTCTGTGAGGGATGATTAGG-3’	481 b.p
Reverse	5’-GCAGACAGGCTAACCACACAC-3’	

IL-12 cDNA

NCBI Reference Sequence: NM_005535.1		
IL-12RB1 Forward	5’- TGAACCTCGCAGGTGGCAGA -3’	2083 b.p
IL-12RB1 Reverse	5’- TCGGGCGAGTCACTCACCCT -3’	
NCBI Reference Sequence: NM_001559.2		
IL-12RB2 Forward	5’- GGCGACACGTGGAAGAATAC -3’	2729 b.p
IL-12RB2 Reverse	5’- AGAGATGACAGCTGCTGGAG -3’	

IKBKG cDNA

NCBI Reference Sequence: NM_003639.3		
Forward	5’- CTGCGCTCTATCGAGGTCGTTAA -3’	1402 b.p
Reverse	5’- AGGAAAGCGCAGACTGCACGGT -3’	

CYBB cDNA

NCBI Reference Sequence: NM_000397.3		
CYBB-1-1 Forward	5’- TGCCACCATGGGGAACTGGGCTGTGAATGAG -3’	628 b.p
CYBB-1-2 Reverse	5’- GTACCAAAAGACTTCAAAGTAAGACCTCCGGATG -3’	
CYBB-2-1 Forward	5’- TGTTGGCAGGCATCACTGGAGTTGTCATCACGC -3’	680 b.p
CYBB-2-2 Reverse	5’- GAACACATCTTCACTGGCAGTGCCAAAGGGCCCATC -3’	
CYBB-3-1 Forward	5’- AATGCTTGTGGCTGTGATAAGCAGGAGTTTCAA -3’	674 b.p
CYBB-3-2 Reverse	5’- AGCATTATTTGAGCATTTGGCAGCACAACCCACA -3’	

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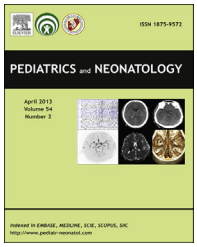

CASE REPORT

Successful Unrelated Cord Blood Stem Cell Transplantation in an X-linked Chronic Granulomatous Disease Patient with Disseminated BCG-induced Infection

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Key Words
chronic granulomatous disease (CGD);
disseminated Bacillus Calmette–Guérin (BCG) infection;
umbilical cord blood transplantation (UCBT)

A 19-month-old boy with chronic granulomatous disease (CGD) received umbilical cord blood transplantation (UCBT) from an unrelated donor after experiencing a life-threatening disseminated Bacillus Calmette–Guérin infection. After busulfan and cyclophosphamide conditioning, we performed a 5/6-matched UCBT. Engraftment and mixed chimerism was 100% in peripheral blood, and 100% of his neutrophils had normal oxidative burst activity on day 17. The patient is now 3 years old, free from infection, and growing well. To our knowledge, this is the second case of CGD treated with UCBT in Taiwan. His successful outcome illustrates that UCBT in a patient with CGD should be considered early if a human leukocyte antigen-matched donor is not available or the patient has just recovered from a severe infection.

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1. Introduction

Chronic granulomatous disease (CGD) is a rare inherited disorder characterized by abnormal functioning of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in phagocyte cells owing to a defect in oxidative killing. Patients may experience recurrent life-threatening bacterial and fungal infections. Despite conventional treatment with prophylactic antibiotic drugs or with interferon gamma, the long-term survival rate remains poor. Only 50% of patients reach the age of 30 years.[1] Umbilical cord blood transplantation (UCBT) from unrelated donors has been successfully used in children with CGD.[2] However, there is limited experience of UCBT in CGD patients, and even less knowledge about its complete clinical course.

2. Case Report

2.1. Past and family history

The boy was first admitted to the hospital at 6 months of age because of a spiking temperature for the previous 14 days and multiple small wounds (dimensions, 2 × 2 cm) with yellowish discharge on the posterior side of the left thigh, the anterior side of the right lower leg, the left shoulder, and at the Bacillus Calmette—Guérin (BCG) inoculation site (Figure 1A—D). His medical history revealed localized axillary lymphadenopathy after a BCG inoculation in infancy that was under regular antibiotic control. He was the fourth child born to nonconsanguineous parents; his two elder brothers had died in early infancy from an unexpected rapid course of sepsis. The patient's body weight was 6.2 kg ($<25^{th}$ percentile), and his height was 64 cm ($<10^{th}$ percentile), and there was no retardation in gross or fine motor skills or speech development.

Physical examination revealed an elastic, mobile, left axillary lymph node (3 × 3 × 2 cm), with no tenderness upon palpation and without overlying erythema. On auscultation, coarse and decreasing breathing sounds with a fine crackle in the right lung fields were audible. The liver and spleen were enlarged, tender, and descended by 4 cm and 3 cm, respectively. A chest radiograph (Figure 1E and F) revealed pleural effusion and a large consolidation with air bronchogram in the right lung, and enlarged left axillary lymph nodes with necrotic change. A magnetic resonance imaging scan (Figure 1G and H) revealed osteomyelitis affecting the left humerus associated with septic arthritis of the elbow joint and the left distal tibia.

Laboratory investigation results were as follows: leukocyte count, 16 × $10^3/\mu L$ (52.6% neutrophils, 8.3% monocytes, and 37.0% lymphocytes); hemoglobin level, 11.1 g/dL; and platelet count, 474 × $10^3/\mu L$. Because his oxidative killing level [measured by flow cytometry with dihydrorhodamine-123 (DHR-123, Invitrogen, Eugene, USA) oxidation analysis] was 0%, he was screened for genetic mutations using polymerase chain reaction and direct sequencing. Five subunits of the reduced NADPH oxidase complex were checked: the X-linked *CYBB*, which encodes gp91-phox, and autosomal p22-phox, p47-phox, p67-phox, and p40-phox. A homozygous c.665A>G (p.H222R) mutation was noted in exon 6 of the *CYBB* gene (Figure 2), confirming an X-linked CGD with gp91-phox deficiency.

2.2. Present history

At 19 months of age, the patient had already received therapy with voriconazole for more than 1 year to prevent

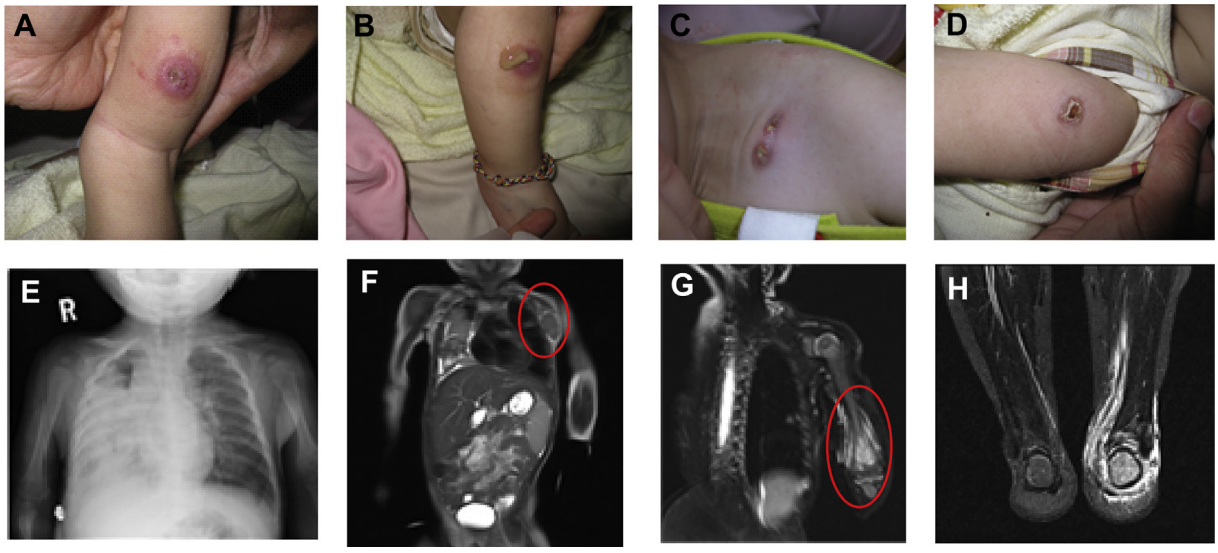

Figure 1 Multiple small wounds with yellowish discharge on the posterior side of (A) the left thigh, (B) the anterior side of the right lower leg, (C) the left shoulder, and (D) the BCG inoculation area. (E) Diffused interstitial fluffy opaque shadow of bilateral parahilar areas with peribronchial thickening and overinflation of both lungs. (F) Enlarged left axillary lymph nodes (3 × 2.4 × 1.9 cm) with necrotic areas and enlarged left cervical and right mediastinal and bronchial lymph nodes. (G) Osteomyelitis on the distal third diaphysis and metaphysis of the left humerus. (H) Marrow cavity of the calcaneus with focal cortical destruction with increased signal along the plantar fascia, the deep fascia of the ankle joint, and the perivascular sheath of the lower leg indicates osteomyelitis. BCG = bacillus Calmette—Guérin.

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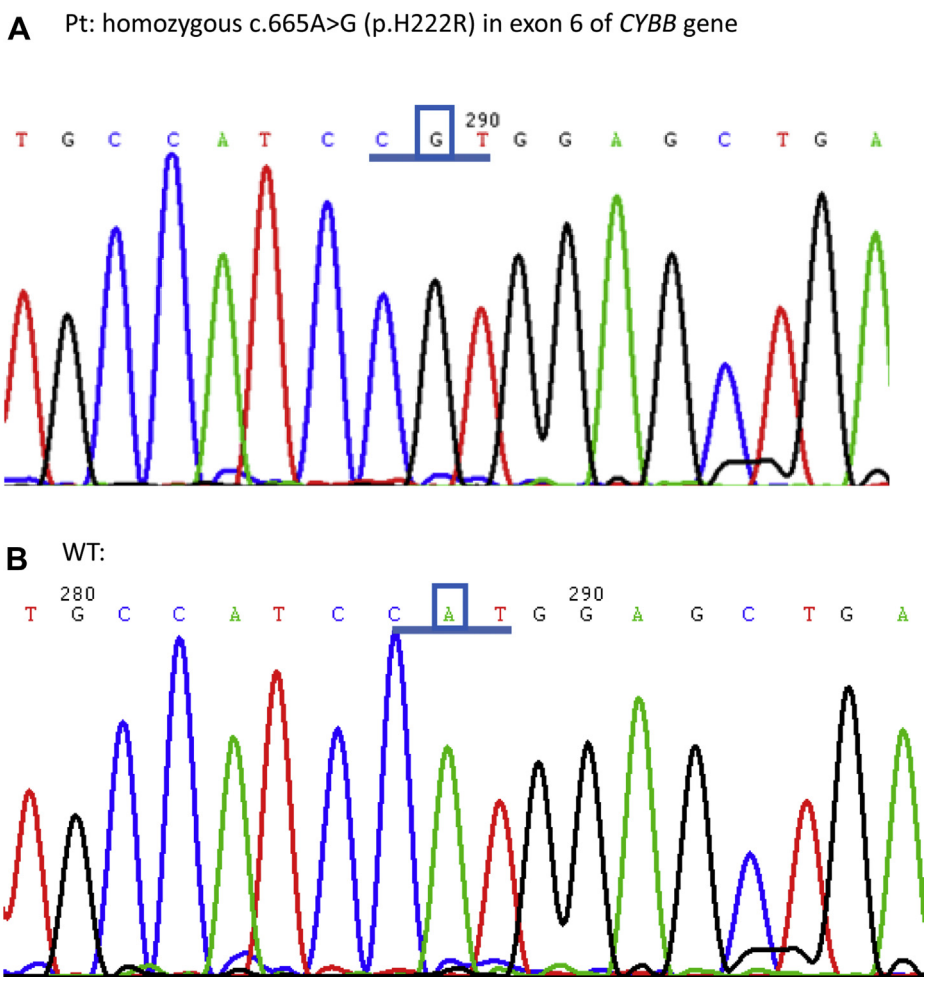

Figure 2 The subunits of the reduced NADPH oxidase complex were performed by polymerase chain reaction and direct sequencing. The c.665A>G (p.H222R) mutation of the *CYBB* gene in the patient (Pt; A) was compared with the presentation in the normal population (WT; B) and CGD was confirmed. CGD = chronic granulomatous disease; NADPH = nicotinamide adenine dinucleotide phosphate.

aspergillosis, which is the most common infection in CGD patients, and ciprofloxacin for suspected BCG-associated or other bacterial infections. Because no clinical evidence of active infection was noted in repeated blood cultures and images, the risks and benefits of hematopoietic stem cell transplantation were discussed. The parents decided to proceed with UCBT because his human leukocyte antigen (HLA) did not match with any of his siblings. An unrelated HLA-A allele-mismatched cord blood unit was obtained from the BIONET Cord Blood Bank, Taipei, Taiwan. The HLA types of the patient were A1101, 3101; B3501, 4001; DRB10101, and 1101. The HLA types of the donor were A1101, 1101; B3501, 4001; DRB10101, 1101. The blood type of the donor was O, and that of the recipient was B. The preparative regimens consisted of 3.5 mg/kg/day busulfan (days −9 to −6), 50 mg/kg/day cyclophosphamide (days −5 to −2), and 3 mg/kg/day rabbit antithymocyte globulin (Thymoglobulin, Genzyme; days −4 to −1). Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine A from day −3 and a short course of methylprednisolone (1 mg/kg intravenously every 12 hours on days 17−18 that was tapered by 25% every day thereafter). The patient underwent UCBT with total nucleated cells exceeding 14×10^7/kg, cell viability exceeding 99.62%, and CD34+ cells exceeding 7×10^5/kg. Sustained engraftment of neutrophils exceeded $0.5 \times 10^3/\mu L$ on day 11 and exceeded $1 \times 10^3/\mu L$ on day 16. The self-sustained platelet count exceeded $20 \times 10^3/\mu L$ on day 24 (October 20, 2011) and exceeded $50 \times 10^3/\mu L$ on day 58 (November 23, 2011). Polymerase chain reaction-based chimerism analysis of peripheral blood revealed complete donor-derived hematopoietic chimerism on day 31 (October 27, 2011). The posttransplant course was complicated by mild fever, and using anticytomegalovirus monoclonal antibody (Mitsubishi, Tokyo, Japan), cytomegalovirus was detected in the patient's plasma. Intravenous ganciclovir was initiated. Grade 1 acute GVHD involving the skin only was resolved with oral methylprednisolone. The cyclosporine A dose was gradually reduced after day 180. Voriconazole and ciprofloxacin were discontinued 3 months after UCBT, and repeat DHR-123 results (Figure 3) indicated normal activity in all the neutrophils after transplantation. The patient is currently free

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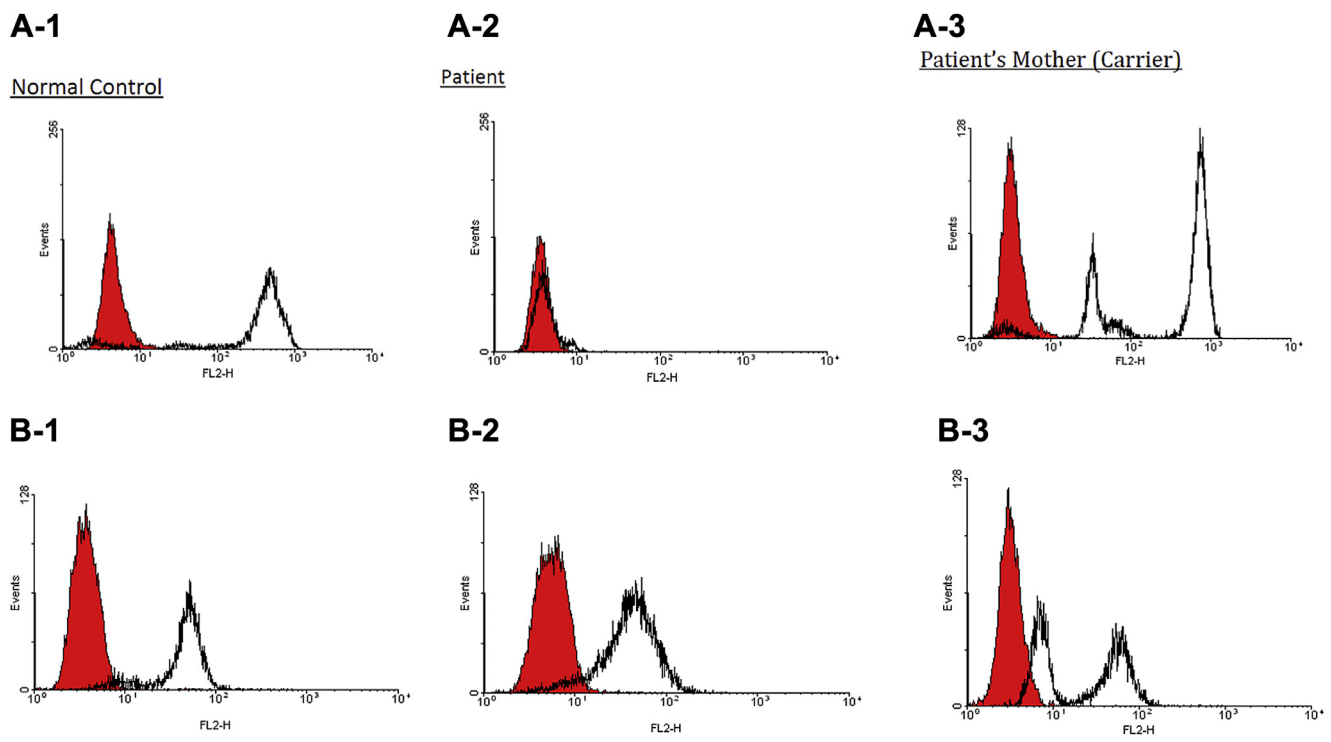

Figure 3 Dihydrorhodamine-123 (DHR-123) test performed twice before (A series) and after (B series) the patient received cord blood transplantation. Neutrophils were incubated with DHR-123 and then activated with phorbol myristate acetate. Preactivation histograms are shown on the left; postactivation histograms are on the right. Blocks A-1 and B-1 show a normal control sample with a large rightward shift in mean fluorescence intensity. Block A-2 depicts the patient with X-linked CGD lacking a detectable oxidative burst. Blocks A-3 and B-3 depict the mother of the affected patient with two populations of neutrophils—one normal and one with mutated gp91-phox. The histogram in Block B-2 shows the patient neutrophil activity shift to the typical pattern observed in the normal control after cord blood transplantation. CGD = chronic granulomatous disease.

from infection and maintains complete donor chimerism without chronic GVHD.

3. Discussion

Two-thirds of all CGD patients possess a mutation in the X-linked *CYBB* gene that encodes gp91-phox. Patients with X-linked CGD have early disease onset and poor prognosis despite antibiotics or interferon gamma use. The annual mortality rate is 5% according to the US CGD registry.[3] Our patient belonged to the high-risk group as he presented with serious pulmonary infections, suspected systemic mycobacterium infection, and osteomyelitis in infancy. In view of poor long-term prognosis and impaired quality of life of CGD patients, bone marrow transplantation (BMT) should be considered at an early stage.[4] However, we used umbilical cord blood instead of BMT because the patient had no HLA-matched siblings and because his sisters were probable carriers of X-linked CGD. In addition, UCBT was more appropriate for his small size. Tomohiro et al[5] performed UCBT in seven CGD children. However, a low survival rate (3/7) was reported, which was probably because UCBT was selected in five of the patients after the BMT had failed, and these patients died from severe bacterial or fungal (aspergillosis) infections. The disadvantages of UCBT include slow hematopoietic reconstitution and graft failure, but these are mostly observed in patients with malignant disorders than those with benign disease.[6] Another study showed that UCBT can be curative in CGD patients with rapidly accessible cord blood and can result in a low incidence and lower severity of GVHD.[7] In Taiwan, 13 children (12 male and 1 female) were diagnosed with CGD (at different hospitals) from January 1985 to December 2010; among these patients, the disease courses of eight children have been described.[8–15] Genetic analysis revealed that two of them had a deficiency of gp91-phox or p47-phox. In five patients, X-linked CGD was suspected because of a previous family history of an affected male sibling or because of a finding of $<$90% nitroblue tetrazolium-positive neutrophils in their mothers. Only one patient underwent successful UCBT,[13] showing good engraftment, rapid hematological recovery, and only mild acute GVHD involving the skin.

BCG vaccine is administered worldwide and can have rare adverse reactions. However, localized axillary lymphadenopathy after a BCG inoculation developed in this male patient in infancy. Attenuated BCG vaccine complications are common in patients with primary immunodeficiency diseases (PIDs), especially those with profound T cell deficiency and CGD. Patients with severe combined T cell and B cell immunodeficiency are suitable for stem cell transplantation. First, this male infant underwent immunological evaluations while he had BCG-related diseases. Second, his family history of two elder siblings' mortality suggested to

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the physicians (obstetricians and neonatologists) that the child might have an X-linked PID. Thus, prenatal diagnosis can be made on the basis of family history, and attenuated vaccine should be avoided in children in such families. All maternal sisters with a family history of X-linked PIDs should therefore undergo genetic analysis to confirm whether they are carriers to enable prenatal diagnosis of their offspring. Two studies[16,17] reported persistent local BCG lymphadenitis as the initial presentation in CGD patients. Very few studies have published evidence about whether the incidence of vaccine strain BCG-induced tuberculosis is high in CGD patients. The paucity of evidence reflects an ascertainment bias because tuberculosis is most prevalent in developing countries where CGD may not be diagnosed.

In conclusion, disseminated mycobacterial BCG infection is often fatal and results from impaired immunity. We believe that UCBT is a potential alternative treatment strategy and may be beneficial for X-linked CGD patients. Rapid immune reconstitution in CGD patients is particularly important when recurrent, ongoing life-threatening infections and irreversible organ damage are likely.

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