Genetic and non-genetic factors affecting the visual outcome of ocular Behçet's disease

Kang E.H. and Song Y.W.
Seoul National University College of Medicine, Korea

We examined the prognostic factors for visual outcome in Korean BD patients with uveitis. Seventy-seven Korean BD patients with uveitis were enrolled. *HLA-B* and *HLA-A* genotypes were determined by PCR-based method. Visual acuity was measured by Snellen chart. Vision loss was graded into visual impairment (VI) defined as VA<20/40 for more than 6 months, loss of useful vision (LUV) as VA<20/200, and near total blindness (NTB) as VA of light perception or worse.

VI was associated with a longer duration of uveitis, posterior uveitis, and cataract, LUV with male gender, a longer duration of uveitis, posterior uveitis, and cataract, and NTB with a longer duration of uveitis, cataract, and glaucoma. *HLA-B*51* and *HLA-A*26:01* did not show any association with VI, LUV, or NTB. However, *HLA-B*51* carriers had earlier onset of uveitis and *HLA-A*26:01* was strongly associated with posterior uveitis. In patients with posterior uveitis, VI was associated with a longer duration of uveitis and cataract, LUV with a longer duration, and NTB with *HLA-B*51*.

In conclusion, longer duration of uveitis, posterior uveitis, male gender, cataract, and glaucoma were found to be associated with poor visual outcome in BD-related uveitis. *HLA-B*51* was associated with NTB in patients with posterior uveitis. *HLA-A*26:01* showed no association with VI, LUV, or NTB, however, was strongly associated with posterior uveitis.

KEY WORDS: Behcet's disease, uveitis, *HLA-B*51, HLA-A*26:01*

High density genotyping of immune-related disease genes identifies new susceptibility loci for Behçet's disease

Masaki Takeuchi[1,2], Nobuhisa Mizuki[1], Akira Meguro[1], Michael J. Ombrello[2], Colleen Satorius[2], Yohei Kirino[1], Tatsukata Kawagoe[1], Duran Ustek[3], Ilknur Tugal-tutkun[3], Emire Seyahi[3], Yilmaz Ozyazgan[6], Shigeaki Ohno[4], Atsuhisa Ueda[1], Yoshiaki Ishigatsubo[1], Ahmet Gül[3], Daniel L. Kastner[2] and Elaine Remmers[2]

1 Yokohama City University Graduate School, 2 National Institutes of Health,
3 Istanbul University, 4 Hokkaido University Graduate School of Medicine

Background/Purpose:

Genome-wide association studies have revealed susceptibility genes for many genetically complex diseases. The Immunochip is a custom array with 196,524 markers in 186 loci selected from analysis of 12 autoimmune diseases. Although HLA-B*51, IL10, IL23R, CCR1, STAT4, KLRC4, and ERAP1 have been reported to be susceptibility genes in previous studies, the pathogenesis of Behçet's disease (BD) remains unclear. The purpose of this study was to perform dense genotyping of loci associated with immune diseases to identify novel susceptibility loci for BD.

Methods:

In this study, 2014 Turkish BD patients and 1826 controls were densely genotyped using the Immunochip. Association analysis was performed after quality control. For novel loci with association test P value $< 5\times10\text{-}6$, additional SNPs in the region were imputed using 1000 Genomes data as the reference panel.

Results:

The basic allele association test confirmed 2 loci, IL10 and CCR1, previously associated with BD and identified 4 novel loci, IL1A-IL1B, SCHIP1-IL12A, IRF8, and PTPN1, which exceeded genome-wide significance. In addition, the FUT2 locus showed dominant model genome-wide significance in the three model genotypic analysis. Imputation data provided an additional novel locus with genome-wide significance, EGR2.

Conclusion:

This immunochip dense-genotyping study identified 6 new BD susceptibility loci. These results have greatly expanded the list of genes with common variants that influence BD susceptibility.

「高密度ジェノタイピングによる新たなベーチェット病疾患感受性遺伝子の同定」

竹内正樹[1,2]、水木信久[1]、目黒　明[1]、Michael J. Ombrello[2]、Colleen Satorius[2]、桐野洋平[3]、河越龍方[1]、Duran Ustek[4]、Ilknur Tugal-tutkun[4]、Emire Seyahi[4]、Yilmaz Ozyazgan[4]、大野重昭[5]、上田敦久[3]、石ヶ坪良明[3]、Ahmet Gül[4]、Daniel L. Kastner[2]、Elaine Remmers[2]

1 横浜市立大学眼科、2 アメリカ国立衛生研究所、3 横浜市大病態免疫制御内科学、4 イスタンブール大学、 5 北海道大学眼科

背景、目的：近年のゲノムワイド関連解析（GWAS）により、多くの疾患で感受性遺伝子が同定されてきている。ImmunoChipは12種類の自己免疫関連疾患のGWASデータをもとに186遺伝子領域に位置する196,524個のマーカーをデザインしたカスタムアレイであり、ImmunoChipを用いることで免疫疾患に関連した遺伝子領域の高密度なジェノタイピングを可能にする。ベーチェット病の感受性遺伝子として、HLA-B*51、IL10、IL23R、CCR1、STAT4、KLRC4、ERAP1などが報告されているがベーチェット病の病態の全容は未だ解明されていない。今回、我々はImmunoChipを用いて、ベーチェット病の新たな感受性遺伝子の同定を試みた。

方法：トルコ人ベーチェット病患者2014名、健常人1826名をImmunoChipを用いて高密度なジェノタイピングを行った。call rate > 0.95を満たすサンプル、およびcall rate > 0.95、マイナーアリル頻度 > 0.01、Hardy-Weinberg equilibrium P 値 > 0.00001 を満たすSNPを解析の対象とした。Identity by descent テストにより、血縁関係のサンプルを除外し（pi-hat > 0.18)、集団の階層化を主成分分析を用いて評価した。関連解析でP値< 5×10-6を示す遺伝子領域については、1000 Genomes dataをもとにimputationを行った。得られたマーカーのうちinfo > 0.8を満たすものを対象に関連解析を行った。basic allele test analysisおよびthree model genotypic analysisにおいて、それぞれP値 < 5×10-8およびP値 < 1.67×10-8をgenome-wide significance（統計学的有意）とした。

結果：basic allele test analysisにより、以前に報告されているIL10、CCR1、および新たに4つの遺伝子領域（IL1A-IL1B、SCHIP1-IL12A、IRF8、PTPN1）においてgenome-wide significanceを越える有意性が見られた。また、FUT2領域が優性モデル（dominant model）でgenome-wide significanceを示した。さらにImputationにより、EGR2領域が新たにgenome-wide significanceを示した。

結語：ImmunoChipによる高密度ジェノタイピングにより新たに6つのベーチェット病感受性遺伝子を同定した。

Treatment of Uveitis Attacks in Behçet's Disease

Yu H.G., Park U. C., and Shin J.
Seoul National University College of Medicine, Korea

Local treatment is clinically relevant for the treatment of uveitis, especially unilateral or asymmetrical cases. We evaluated the long-term efficacy and safety of intravitreal triamcinolone acetonide (IVTA) injection for posterior segment inflammation in Behçet's disease (BD) patients. Forty-nine patients (49 eyes) were included. Mean best-corrected visual acuity improved from 0.89 logMAR units to 0.70, 0.64 at 12, 24 months, respectively. Complete inflammation control was achieved in 87.0% of patients, but 60.0% of them experienced relapse within 12 months. For phakic eyes, cumulative probabilities for cataract surgery were 13.8%, 48.9%, and 60.2% at 12, 24, and 36 months, respectively. Intraocular pressure elevation exceeding 21 mmHg was noted in 40.8%. Therefore, we concluded that in Behçet uveitis attack that is unresponsive or intolerant to systemic medications, IVTA injection is an effective therapeutic option, although ocular complications could limit its efficacy and repeatability. Also, in this talk, we are going to introduce a new randomized clinical trial of intravitreal triamcinolone acetonide injection for the treatment of endogenous uveitis.

Oral Streptococcus is one of immunopathological triggering factors in aphthous ulceration including Behçet's disease

Fumio Kaneko[1], Ari Togashi[1], Erika Nomura[1] and Koichiro Nakamura[2]
1 Institute of Dermato-Immunology and Allergy, Southern TOHOKU Research Institute for Neuroscience, Koriyama and 2 Department of Dermatology, Saitama Medical University, Saitama, Japan

There may be some difficulties to differentiate Behcet's disease (BD) and recurrent aphthous stomatitis (RAS) from other aphthous lesions including herpes simplex virus (HSV) infection. "Pathergy test" has been thought as one of auxiliary diagnostic benefits for BD for long time, albeit less sensitivity. The prick reaction by streptococcal antigens is highly diagnostic for BD patients though the standard antigens are unable to be routinely provided, because the patients have hyper-reactivity against streptococci. More simply specific diagnostic ways should be considered. The prick with neat and filter-sterilized self-saliva (S- and SS-prick) was performed on the skin of the patients, because oral streptococci are contained in saliva. The skin reactions were measured in diameter 48 hours after prick and the biopsy was performed from the skin reaction to analyze immunohistologically.

In 9 out of 10 BD patients (90%), the S-prick reaction was intensely observed and 3 out of 5 RAS (non-BD) patients (60%) showed relatively weak reaction. The reactive severity was not correlated with HLA-B51 in BD patients. None of other patients and healthy controls responded to the prick. As no reaction appeared by the SS-prick, the response by S-prick might be due to oral streptococci in the patients. Salivary culture revealed streptococcal colonies in MS agar. Histology of the pricked skin-site displayed predominant infiltration of CD4+T cells and monocyte/macrophage lineage resembling to the vascular reaction of erythema nodosum-like eruption in BD patients.

The S-prick is helpful in diagnosis for BD and RAS. The results suggest that the positive pathergy reaction of BD patients might be due to cutaneous microorganisms having cross-immunity to oral streptococci and that may provide a direct evidence for triggering action in the underlying immunopathology of the two oral disease conditions.

「口腔内連鎖球菌はベーチェット病を含むアフタ性潰瘍を惹起する免疫病態機序の一因である」

金子史男[1)]、富樫亜吏[1)]、野村絵里香[1)]、中村晃一郎[2)]
1）脳神経疾患研究所付属皮膚免疫・アレルギー疾患研究所、2）埼玉医科大皮膚科

ベーチェット病（BD)、再発性アフタ性口内炎（RAS）と単純ヘルペスによる口腔内アフタ病変の鑑別はしばしば困難である。長い間、針反応がBDの補助診断に有用と考えられてきたが、その特異性は乏しい。BD患者は連鎖菌に過敏反応を示すことから、その菌体抗原によるプリック試験は診断に有用だが、標準抗原の安定供給は困難である。そこで、より簡便化した診断法を検討した。唾液には口腔内連鎖球菌が含まれているので、自家唾液とそのフィルター滅菌した唾液と生食によるコントロールによるプリック試験（S-,SS-およびCSプリック）を施行した。皮膚反応は48時間後にその長径を測定して判定し、併せて同部位の生検を免疫組織学的に解析した。

BD10例中9例（90％）で強いS-プリック反応が、RAS（非BD患者）では比較的弱い反応が5例中3例（60％）に観察された。BD患者ではその反応強度とHLA-B51に相関はなかった。そのほかの患者、健常者では反応は見られなかった。SS-プリックでは全く反応がなかったことから、連鎖球菌の関与が考えられ、実際、唾液培養ではMS寒天培地上に連鎖球菌が検出される。BD患者では、組織学的に結節性紅斑の血管反応に類似したCD4陽性細胞と単球系細胞主体の浸潤がみられた。

S-プリック反応はBDおよびRASの診断に有用であった。この結果は、BD患者における針反応陽性は、口腔内連鎖球菌と免疫学的に交差反応性をもつ皮膚微生物により生じたものである可能性があること、これらの疾患の口腔内病変の免疫病態は全身症状発現機序を示唆している。

The role of memory T cells in a Behçet's disease mouse model

Choi B., Lee E.S., Sohn S.
Ajou University School of Medicine, Korea

According to a previous report, thalidomide treatment resulted in increased frequencies of CD4+CD45RO+ memory T cells in Behçet's disease (BD) patients. The relationship between active symptoms and CD8+ memory T cells is needed to be elucidated. To clarify this relation, the frequencies of CD8+ memory T cells were analyzed and modulated in a BD mouse model. The BD mice used were developed by inoculation on the scratched earlobe with Herpes Simplex Virus (HSV). The frequencies of CD8+CD44+ memory T cells and CD8+CD62L- memory T cells were lower in BD mice than in no symptomatic control mice. In accordance with the changes of frequencies of CD8+CD44+ memory T cells and CD8+CD62L- memory T cells by the treatment of Polyinosinic:polycytidylic acid (Poly IC) or CCL21 expression vector, BD-like symptoms of mice were also affected. Concurrently, the frequencies of CD8+CD122+ T cells and serum levels of IL-10 and IL-17A were modulated when compared with control groups. These findings suggest the expression level of CD8+ memory T cells were regulated by immune modulators and these are correlated with the regulation of BD-like symptoms in a HSV induced BD mouse model.

IL-12 family cytokine involvement in helper T cell differentiation in patients with Behcet's disease (BD)

Noboru Suzuki[1], Jun Shimizu[1], Fumio Kaneko[2], Sakae Kaneko[3].

1 Department of Immunology and Medicine, St. Marianna University School of Medicine, Japan

2 Department of Dermatology, Fukushima Medical University, Fukushima, Japan

3 Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan

We have presented evidences that high frequency of helper T (Th) 17 cells and overactivity of Th cells against both IL-12 and IL-23 in patients with BD. Recently, some researchers revealed that IL-12, IL-23, IL-27 and IL-35 are heterodimeric and share the subunits, and named them IL-12 family cytokines. It is thought that the 4 cytokines have overlapping but distinct effects to T cells with corresponding Janus kinase (JAK)-STAT signaling pathway. The experimental data demonstrated a functional spectrum from proinflammatory to inhibitory in Th cell differentiation. This investigation was designed to study the functional differences of IL-12 family cytokines in Th cell differentiation between BD and normal controls.

We cultured Th cells with IL-12 family cytokines and evaluated cytokine production and gene expression of the cells obtained from patients with BD (n=5) and NC (n=4). In patients with BD, high frequency of IL-17 producing Th17 cells was observed in the presence of IL-23 and anti-IL-23 (p19) compared to NC ($p<0.05$). On the contrary, frequency of IFNγ producing Th1 cells was significantly low in the presence of IL-23, IL-35, anti-IL-23 and a JAK inhibitor (P6) in patients with BD compared to NC ($p<0.05$). Gene expression of IFNγ in two patients with BD was relatively high in case of IL-12, IL-23 and IL-35 stimulation but the average value showed no significant difference between BD and NC.

These results suggest that skewed Th cell differentiation in patients with BD against IL-12 family cytokines is assessed by in vitro experiments.

「ベーチェット病CD4+T細胞分化におけるIL-12ファミリーサイトカインの関与」

○鈴木　登（聖マリアンナ医大　免疫学・病害動物学）
清水　潤（聖マリアンナ医大　免疫学・病害動物学）
金子史男（福島県立医大　皮膚科）
金子　栄（島根大医学部　皮膚科）

【目的】われわれは昨年の本会議において、ベーチェット病（BD）の病態におけるTh17型イフェクターT細胞の増加と、その増加にIL-23による過剰刺激が関与する可能性があることを報告した。近年Th1細胞分化に必須であるIL-12とIL-23はIL-27、IL-35は、それぞれの受容体および細胞シグナルに多くの共有部分が存在することよりIL-12ファミリーと称されるようになってきている。このファミリーのT細胞分化に与える影響は、炎症惹起から抑制まで多岐にわたることも徐々に明らかになってきた。そこで今回の報告では前回に引き続き、このサイトカインファミリーによるヘルパーT細胞分化制御を試みたので報告する。
【方法および結果】患者（n=5）および健常人（n=4）よりメモリーヘルパーT細胞を採取。IL-12、IL-23、IL-35、抗IL-23抗体（p19）およびJAK阻害剤の存在下で培養し、IL-23受容体発現量、IFNγおよびIL-17の産生量をフローサイトメーターで観察。さらにTh17関連遺伝子発現を、リアルタイムPCRで観察した。統計処理はWilcoxon検定を用い、$p<0.05$で有意とした。

今回の検討では細胞内サイトカイン産生に関しては健常人に比較し、それぞれの刺激でTh17細胞頻度は上昇傾向にあったが、IL-23および抗IL-23抗体の存在下で有意差を持った。一方、Th1細胞はIL-23、IL-35、抗IL-23抗体、JAK阻害剤の存在で有意に減少した。
またTh17関連遺伝子発現では、IFNγ遺伝子発現がBD2症例のIL-12、IL-23、IL-35との培養での上昇が観察されたが、発現値の平均では健常人との間に有意差は認めていない。
【結論】BDヘルパーTリンパ球においてIL-12ファミリーの入出力異常が存在し、ビトロでも確認されたと推察する。

Changing clinical expression of Behçet's disease in Korea during three decades (1983-2012)

Kim D.Y.,[1] Choi M.J.,[1] Cho S.,[1] Lee E.-S.,[2] Lee S.,[3] and Bang D.[1]

1 Yonsei University College of Medicine, Korea

2 Ajou University School of Medicine, Korea

3 Gachon University Graduate School of Medicine, Korea

Background: Behçet's disease (BD) is a chronic multisystemic vasculitis affecting blood vessels of any caliber or type. Recent evidence suggests that the clinical expression of BD is lessening.

Objectives: Our aim was to examine the clinical expression of BD in Korea during the past three decades via a large patient registry.

Method: Initial manifestations of patients with BD seen at a tertiary referral hospital from 1983 to 2012 were reviewed retrospectively, stratifying patients by decade to compare epidemiologic data and cardinal symptoms.

Results: A total of 3,674 patients with BD were reviewed. Significant proportionate declines occurred with respect to male gender, complete type of BD, and major presenting features (genital ulcers, ocular involvement, and skin lesions) whereas mean patient age rose progressively, and joint, gastrointestinal (GI), and central nervous system (CNS) symptoms increased.

Conclusion: During the past three decades, clinical expression of BD in Korea has changed, resulting in fewer instances of complete type disease, declining male propensity, and shifting patterns of organ involvement.

Ethnic and clinical features of Behçet's disease in Russia

Kitaichi N[1,2], Lennikov A[3], Alekberova Z[4], Namba K[2], Takeno M[5], Mizuki N[5], Ishigatsubo Y[5], Ohno S[2]

1 Health Sciences University of Hokkaido, Sapporo, Japan
2 Hokkaido University Graduate School of Medicine, Sapporo, Japan
3 Sapporo Medical University, Sapporo, Japan
4 Russian Academy of Medical Science, Moscow, Russia
5 Yokohama City University Graduate School of Medicine, Yokohama, Japan

Clinical features of Behçet's disease (BD) are well described in European and Asian countries, however the Russia and Caucasus area still has only a limited amount of information regarding the disease. In the present study, 250 consecutive patients diagnosed with BD between 1990 and 2010 at the Research Institute of Rheumatology, Russian Academy of Medical Sciences (RAMS) in Moscow were enrolled. The ethnic backgrounds of the patients were reported as follows: 23.2% (58 cases) from Russia, 21.6% (55 cases) from Dagestan, 14.4% (36 cases) from Armenia, 12.8% (32 cases) from Azerbaijan, and 8.8% (22 cases) from Chechnya. The remaining 19.2% (48 cases) were from other regions or of unknown origin. More than a half (57.6%) of the Behcet's disease patients originated from Central Asia, specifically Azerbaijan, Armenia, Chechnya, and Dagestan. The mean age at disease onset was 31.5 years old, and the most typical initial manifestations were oral aphthous ulcers. The manifestations observed throughout the course of the disease included oral aphthous ulcers (100%), various cutaneous lesions (88.8%), genital ulcers (81.2%), and ocular lesions (54.0%). Involvements of ocular ($p<0.01$) and skin ($p<0.01$) lesions were more frequent in men than in women. HLA-B51 was found in 63.0% of BD patients compared to 20.7% of the healthy control subjects ($p<0.001$), and HLA-A26 was present in 11.3% of BD patients and 18.9% of the control group.

This study shows the presence of BD in Russia. People in Dagestan, Armenia, Azerbaijan and Chechnya consist only 1.7%, 3.0%, 5.3%, and 1.0% of the Russian population, respectively. It is suggested that its prevalence in Central Asian people is much higher than that in White Russian.

「ロシアにおけるベーチェット病臨床像の検討」

○北市伸義（北海道医療大　眼科）
南場研一（北海道大　眼科）、岳野光洋（横浜市大　病態免疫制御内科学）
水木信久（横浜市大　眼科）、石ヶ坪良明（横浜市大　病態免疫制御内科学）
大野重昭（北海道大　眼科）

ヨーロッパやアジアと比べ、ロシアでのベーチェット病の情報は不十分で臨床像にも不明な点が多い。今回我々はロシア科学アカデミー（RAMS）リウマチ研究所を1990年から2010年前に受診した250人のベーチェット病患者を対象に、その民族的背景や臨床像を検討した。

平均発症年齢は31.5歳であった。主症状では口腔内アフタ性潰瘍が100％、皮膚症状が88.8％、外陰部潰瘍が81.2％、眼症状が54.0％であった。皮膚症状と眼症状は男性に有意に多かった（$P<0.01$）。HLA-B51保有者は患者群で有意に多く見られた（$p<0.001$）。

民族別に集計するとロシア人患者は 23.2％のみであった。一方、21.6％がダゲスタン人、14.4％がアルメニア人、12.8％がアゼルバイジャン人、8.8％がチェチェン人であり、患者の半数以上はシルクロード地域の中央アジア民族出身者であった。

ベーチェット病はロシアにもみられた。ロシア政府の人口統計ではロシア人は全国民の77.7％を占める。一方、ダゲスタン人は1.7％、アルメニア人は3.0％、アゼルバイジャン人は5.3％、チェチェン人は1.0％を占めるにすぎない少数民族であり、中央アジア人は白系ロシア人よりベーチェット病発症率が高いと推測された。