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学 位 論 文 題 名

Seeking for new disease susceptibility genes in Vogt-Koyanagi-Harada disease

(原田病における新しい疾患感受性遺伝子の探求)

学位論文内容の要旨

OBJECTIVES: Vogt-Koyanagi-Harada (VKH) disease is one of the most frequent forms of uveitis in Japan, and is characterized as a panuveitis accompanied by neurological lesions such as headache and pleocytosis of the cerebrospinal fluid, skin lesions such as vitiligo, alopecia, and inner ear disturbances. This disease is considered to be an autoimmune disease against melanocytes. In earlier studies, melanocyte contains tyrosinase (TYR), tyrosinase - related protein (TRP) 1 and TRP2, and lymphocytes separated from VKH disease patients were reactive to peptide fragments derived from these proteins. In experimental animal models, melanocyte - specific proteins, TRP1 and TRP2, induced an experimental autoimmune disease in Lewis rats that resembled human VKH disease. Inflammation induced by TRP1 in Akita dogs also resembled human VKH disease. These studies suggest that TYR, TRP1 and TRP2 are responsible for human VKH disease. Interferon-γ (IFN-γ) is significantly elevated in the aqueous humor and sera of VKH patients. IFN-γ is important cytokine for VKH disease and is associated with the development of Th1-dominant, cell mediated immune responses which may thereby enhance the expression of human leukocyte antigen (HLA) class II antigens. VKH disease is known to be associated with HLA class II antigen, *HLA-DRB1*04* and numerous studies have shown that about 80% of VKH patients have HLA - *DRB1*0405*. However, little is known about the true pathogenic gene related to VKH disease. As for the investigation of disease susceptibility genes, association studies are now primarily conducted with single nucleotide polymorphisms or microsatellites because they are ubiquitous in the genome. Microsatellite (MS) polymorphisms show a greater diversity than single nucleotide polymorphisms (SNPs) and have been widely used in both linkage and association studies of disease. Microsatellite linkage disequilibrium (LD) length is in the approximately 100 kb range when compared with the shorter range for SNPs. Therefore, the advantage of microsatellite analysis is that a collection of relatively small numbers of polymorphic markers can make association analyses an immediate reality. To investigate whether melanocyte containing protein is responsible for VKH disease or not, we analyzed polymorphisms in MSs among *TYR*, *TYRP1* and *TYRP2* locus. The *TYR* gene, *TYRP1* gene and *TYRP2* gene are located on the chromosome 11q14-q21, 9q23 and 13q32, respectively. These genes encode the enzymes involved in melanin formation and are expressed specifically in melanocytes. We speculated that polymorphisms within the *TYR*, *TYRP1* and *TYRP2* may be related to VKH disease. As previously described, these candidate genes encode the enzymes involved in the melanin formation, and are the class of genes that have been associated with depigmentation and ocular developmental defects. In previous studies, mutations of *TYR* and *TYRP1* caused oculocutaneous albinism (OCA) 1, OCA3, and microphthalmia, and *TYRP2* gene caused melanoma and vitiligo. These mutations include missense, nonsense, frameshift, and splice site mutations, and deletion of the entire coding sequence.

The *IFN-γ* gene on the chromosome 12q24.1 spans approximately 5.4 kb and contains four exons. Like other cytokines, the IFN-γ coding region is invariant, with no reported polymorphisms. Single nucleotide polymorphism (SNP; rs2430561) and microsatellite (rs3138557) within the first intron of the *IFN-γ* gene correlate with a high amount of in vitro production of IFN-γ and are associated with disease severity or resistance to drug therapy in various autoimmune diseases. This allele is associated with a higher or a lower risk of a variety of diseases including autoimmune and chronic inflammatory conditions. The association between SNP (rs2430561) alleles T to A with a low (AA), medium (AT), and high (TT) production of cytokines has been reported in vitro. We hypothesized that a common allelic variation in these potential functional polymorphisms may be involved in Th1-mediated autoimmune diseases, such as VKH disease.

In this study of Japanese subjects, we investigated whether the microsatellite or single nucleotide polymorphisms in the *TYR*, *TYRP1*, *TYRP2*, *IFN-γ* gene and *HLA-DRB1* contribute to the development risk of VKH disease and to some of the clinical features of the disease.

METHODS: The study involved totally 136 VKH patients and 176 healthy controls who were genotyped for *HLA-DRB1*, *TYR*, *TYRP1*, *TYRP2* and *IFN-γ*. *HLA-DRB1* genotyping was performed by the PCR-restriction fragment length polymorphism (RFLP) method. Microsatellite (MS) polymorphisms within *TYR*, *TRP1*, *TRP2* and *IFN-γ* and functional single nucleotide polymorphism (SNP) of *IFN-γ* were analyzed. In addition, the haplotype frequencies of the microsatellite in *TYR*, *TRP1* and *TRP2* were also estimated and statistical analysis was performed. Moreover, clinical manifestations of the patients were also analyzed.

RESULTS: The age of the patients ranged from 15 to 80 years (51.9±13.7, mean±SD). The study group included 65 men (47.8%) and 71 women (52.2%). Based on the diagnostic criteria for VKH disease, 30 cases (22.1%) were classified as complete VKH disease, 89 cases (65.4%) as incomplete VKH disease, and 17 cases (12.5%) as probable VKH disease. Both eyes were affected in all patients. Diffuse choroiditis/staining of fluorescein on angiography was observed in all patients. Sunset glow fundus and nummular chorioretinal depigmented scars were observed in 83.9% and 36.1% of the patients, respectively. Neurological and auditory disorders were observed in 90.1% of the patients: meningitis (e.g., headache and fever) in 79.8%, tinnitus in 53.0%, and cerebrospinal fluid pleocytosis in 70.0%. Dermatologic manifestations were observed in 22.9% of the patients: alopecia (6.9%), poliosis (17.6%), and vitiligo (13.0%). There was no significant difference between 87 VKH patients and 122 healthy controls at seven microsatellites within the region of *TYR*, *TYRP1* and *TYRP2*, nor in haplotype frequency. There was no significant difference in the allele and genotype frequencies between 136 VKH patients and 176 healthy controls in the SNP (rs2430561) and microsatellites (rs3138557) of *IFN-γ*. In our clinically stratified analysis, we investigated the presence of some of the clinical features, such as disease onset, presence of diffuse choroiditis, sunset glow fundus, depigmented scars, meningitis, tinnitus, cerebrospinal fluid pleocytosis, and integumentary finding. None of these clinical findings were significantly associated with the microsatellite and SNPs. Then we classified VKH patients according to the diagnostic criteria as complete, incomplete, and probable VKH disease. However, significant association was not detected between any type of VKH disease and healthy controls, neither. The magnitude of LD between SNP (rs2430561) and microsatellite (rs3138557) showed an extremely high value, with pair-wise LD valued at D' >0.96 in controls and >0.99 in the patients. As for the *HLA-DRB1* genotyping of the 87 VKH patients and 122 healthy controls, the phenotype frequency of *HLA-DRB1*04* was significantly increased in VKH patients (n=71, 81.6%) compared to healthy controls (n=53, 43.4%) [Odds ratio (OR) = 5.78, P= 3.08E-8, Pc = 7.70E-8]. Within the *HLA-DRB1*04*, the phenotype frequency of *HLA-DRB1*0405* was remarkably higher in VKH patients (n=61, 70.1%) compared to healthy controls (n=35, 28.7%) (OR = 5.83, P = 3.10E-9, Pc = 7.90E-8).

CONCLUSIONS: We concluded that there is no genetic susceptibility or increased risk attributed to the *TYR*, *TYRP1*, *TYRP2* and *IFN-γ*. *HLA-DRB1*0405* showed a highly significant association as expected. The mutational characterization of genes involved in VKH disease will provide additional insight into the molecular mechanisms underlying this common uveitis in the Japanese population.

学位論文審査の要旨

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学位論文題名

Seeking for new disease susceptibility genes in Vogt-Koyanagi-Harada disease

（原田病における新しい疾患感受性遺伝子の探求）

Vogt-Koyanagi-Harada 病（原田病）は髄膜刺激症状、髄液細胞増多、感音性難聴などの神経障害、白斑、脱毛、白髪などの皮膚障害を伴う両眼性の汎ぶどう膜炎である。この疾患はメラノサイトに対する自己免疫疾患であると考えられている。本研究は、HLA-DR、TYR、TRP1、TRP2 そして IFN-γ遺伝子の多型を原田病患者 136 例と健常人 174 例で比較したものである。北海道大学眼科あるいは横浜市立大学眼科で、原田病と診断された患者の末梢血から DNA を採取し、Primary chain reaction (PCR)、ダレクトシークエンス法、HLA-DRB1 タイピングにより比較検討した。

その結果、TYR、TYRP1,2、IFN-γ遺伝子では、疾患群と健常群の間で有意差はみられなかった。原田病の抗原と考えられる TYR、TYRP1,2 における遺伝子レベルでの違いはないことがわかった。IFN-γ遺伝子についても有意差が見られなかったことから、IFN-γ遺伝子により前房や血中の産生量が増加しているわけではないことがわかった。HLA-DR タイピングの結果、DR4 が患者群で 81.6%、健常対照群で 43.4%と有意に患者群で多くみられた。そのうち特に DRB1*0405 が患者群で 70.1%、健常対照群で 28.7%、相対危険度 5.83、補正 P 値が 7.9×10 のマイナス 8 乗と強い相関がみられた。結論として、HLA-DR4 とくに HLA-DRB1*0405 が原田病患者で有意に多くみられたが、病態と関係すると考えられている TYR、TRP1、TRP2、IFN-γ遺伝子には、原田病患者と健常人とに違いはみられなかった。

学位論文の審査は、平成 21 年 2 月 3 日、主査の本間教授、副査の笠原教授、守内教授の 3 名により、公開審査として医学研究科臨床大講堂で行われた。なお、出席者は約 50 名であった。主査から紹介があった後、申請者はスライドを用いて約 10 分間にわたり、学位論文の内容を発表した。その後、主査、副

査と申請者による質疑応答が約20分間にわたって行われた。笠原教授からは、今回対象とした遺伝子では有意差が認められなかったが、今後どの遺伝子に着目すべきか、原田病で自己免疫が起こる機序、チロシナーゼ、TRP1,2、IFNのコード領域における多型について、守内教授からは、研究試料収集法、人種による臨床症状の相違、病態解明の観点から網羅的なRNA解析の妥当性について、本間教授からは、原田病におけるメラノサイトの抗原部位、本研究以外の病態解明戦略、臨床症状発現における環境因子について、それぞれ質問があった。申請者は、いずれの質問に対しても、学位論文のデーターや過去の文献を引用し、概ね適切に回答した。

本研究は、本邦に多発する原田病の病態を、分子生物学的および遺伝学的手法を用いて解明することを試みたもので、HLA-DR4とくにHLA-DRB1*0405が原田病患者で多くみられることを明らかにした。審査員一同は、これらの成果を高く評価し、大学院課程における研鑽や取得単位なども併せ申請者が博士（医学）の学位を受けるのに充分な資格を有するものと判定した。