厚生労働科学研究費補助金

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若年性特発性関節炎の遺伝的要因の実態

平成２２年度　総括・分担研究報告書

主任研究者　　松本　直通

（横浜市立大学大学院医学研究科環境分子医科学・教授）

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平成21年度厚生労働科学研究費補助金（難治性疾患克服研究事業）
総括研究報告書

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研究代表者　　松本直通　横浜市立大学大学院医学研究科教授

若年性特発性関節炎（juvenile idiopathic arthritis, JIA）は、16歳未満で発症する関節を主病変とする慢性炎症性疾患で、その原因は未解明である。その発症には自己免疫が関与し、小児膠原病としては比較的頻度が高い。発症率は年間10万人に2-20人程度である。2008年に抗IL-6受容体抗体(tolicizumab)が、全身型JIAの症例に著効することが、研究分担者・横田らにより報告された(Lancet, 2008)。本研究は、JIAの遺伝的な原因解明を目的とした基礎・臨床研究を目指して開始、興味深い候補遺伝子Xの明らかな変異を1例で認めたが、他の50症例での追試では遺伝子Xおよびその機能的関連遺伝子Yの変異はなかった。JIAと関連する病的なCNVを探索するため全身型JIA50例に対して高密度マイクロアレー解析を行い両親には認めない約77 Kbと622 Kbの不連続な*de novo*重複を1例に同定した。この重複領域内におよそ30程度の免疫系・炎症系に深く関与する候補遺伝子が包含されJIA責任遺伝子の有力な候補と考えた。これまでに5遺伝子の変異スクリーニングを行うも変異は見出せていない。

分担研究者

横田俊平・横浜市立大学医学部小児科

A．研究目的

若年性特発性関節炎（juvenile idiopathic arthritis, JIA）は、16歳未満で発症する関節を主病変とする慢性炎症性疾患で、その**原因は未解明**である。その発症には自己免疫が関与し、小児膠原病としては比較的頻度が高い。発症率は年間10万人に2-20人程度である。2008年に抗IL-6受容体抗体（tolicizumab）が、全身型JIAの症例に著効することが、研究分担者・横田らにより報告された(Lancet, 2008)。**本研究班は、JIAの遺伝的な原因解明を目的とした基礎・臨床研究を目指して構成されている。**研究代表者・松本は、全身型JIA50例に対して全ゲノムオリゴDNAアレー解析を開始した。その過程で、免疫系において極めて重要な遺伝子Xの13 Kbにわたる遺伝子内欠失を同定した（未発表データ）。本発見がJIAの根本的解明の突破口となる可能性が期待されたが、JIA50例を対象とした遺伝子X及びその機能的関連遺伝子Yの変異解析では変異を同定できず、遺伝子XをJIAの責任遺伝子であると結論づけるに至っていない。新たにJIAと関連する病的なCNVを探索するため全身型JIA50例に対して高密度マイクロアレー解析を行い染色体19番上に両親には認めない約77 Kbと622 Kbの不連続な*de novo*重複を1例に同定した。この重複領域内におよそ30程度の免疫系・炎症系に深く関与する候補遺伝子が包含されJIA責任遺伝子の有力な候補と考えられた。本年度の研究の目的は、遺伝子X以外の候補遺伝子探索とその全身型JIAにおける寄与度を明らかにすることである。

B．研究方法

平成22年
I. 症例集積（横田・松本）

研究分担者横田は、横浜市大に於いて現在、JIA 約 200 例を診療し、平成 21 年度に全身型 JIA で Tolicizumab 治療例 50 例を解析対象とし集積し、現在各症例において末梢血リンパ球から DNA および細胞ペレットの調整とリンパ芽球化が完了している。発症率は年間 10 万人に 2-20 人程度で稀な疾患であるが、集積した検体は詳細な臨床情報が得られる質の高い貴重な研究リソースである。

II. 遺伝子 X の JIA における寄与度を明らかにする(松本)

全身型 JIA 50 例を対象に高密度オリゴ DNA アレーを用いた全ゲノム CNV 解析を開始した。その過程で免疫系において極めて重要な遺伝子 X の 13 Kb にわたる遺伝子内欠失を同定した（未発表データ)。本遺伝子 X の全身型 JIA 症例 50 例における変異解析を行った（研究代表・松本)。変異解析は、High Resolution Melting（HRM）法を用いた。

III. 候補遺伝子アプローチ(松本)

遺伝子 X のコードするタンパク質の機能的関連する遺伝子 Y も JIA の有力な責任候補遺伝子であり候補遺伝子を症例群で解析する。

IV. アレーによる全ゲノム微細構造異常解析(松本)

Affymetrix 250K（全ゲノムに 25 万オリゴ DNA 搭載）または SNP 6.0 (全ゲノムに 185 万のオリゴ DNA 搭載)を用いて、JIA 症例群に対して全ゲノム微細構造異常解析を行う。同定された微細欠失・重複領域はカスタムアレー等で詳細に範囲を決定し、責任候補遺伝子リストを作製し、遺伝子変異探索を行う

V. 関連・責任遺伝子異常と JIA の臨床病型の比較検討(横田・松本)

本研究で明らかになった各関連遺伝子型と JIA 症例の詳細な臨床情報を分析する。関連・責任遺伝子型に対応する臨床病型が明らかとなると期待される。

C．研究結果

I. 症例集積と細胞株化

全身型 JIA50 例の末梢血採取が完了し、DNA および細胞ペレットを調整しリンパ芽球株化も全て終了した。

II. 遺伝子 X の解析

アレー解析を開始したが、その過程で免疫系において極めて重要な遺伝子 X（未発表のため仮称）の 13 Kb にわたる遺伝子内欠失を同定した。家系解析にて患児の父（未発症)、妹（未発症）にも同様の欠失がみられた。非常に興味深いことに遺伝子 X は既に優性遺伝性の A 症候群の責任遺伝子としてミスセンス変異の報告がある。この欠失は *de novo* ではないが病的意義が疑わたるため、患児の末梢血リンパ球由来のリンパ芽球から抽出した mRNA の sequence 解析ではフレームシフト変異による早期終始コドンの出現が確認された。その他の 49 症例に関しても同様にリンパ芽球から抽出した mRNA の変化を観察したが、異常サイズの mRNA が確認されたのは欠失症例のみで他の症例では正常サイズを示した。また症例のリンパ芽球における蛋白質レベルの変化について Western blot を用いた確認作業を進めている。さらに、遺伝子 X の遺伝子内欠失を認めた JIA 患児において、A 症候群に特徴的なリンパ球のアポトーシス障害や TCRα/β 陽性 CD4/CD8 陰性の double negative T cell の増加は認められなかった。このことは、遺伝子 X が A 症候群の責任遺伝子であるとする現在の説を覆す可能性のある極めて重要な知見であると考える。また集積した残りの 49 例を対象に HRM 法を用いて遺伝子 X の変異解析を行ったが、変異は同定されなかった。以上の結果より、今回同定した遺伝子 X の異常が JIA に及ぼす病的意義は小さい（稀）と考えられている。

III. 候補遺伝子アプローチ

遺伝子 X に機能的に関連する Y 遺伝子を候補として、II と同様に HRM 法を用いて変異解析を行ったが、病的な変異は同定されなかった。

IV. アレーによる全ゲノム微細構造異常解析

全身型JIA50例を対象に高密度オリゴDNAマイクロアレー（23名：Affymetrix Genechip Human Mapping 500K array，27名：Genome-wide Human SNP array 6.0）を用いて、全ゲノムレベルの染色体微細構造異常解析を行い詳細について検討した。そして1例において染色体19番上に両親には認めない約77 Kbと622 Kbの不連続な*de novo*重複を認めた（論文投稿中）。この領域内には約30の遺伝子が存在し、免疫系に関与する興味深い遺伝子も多数包含され（他の類縁疾患責任遺伝子と相同性のある遺伝子を含む）、HRM法を用いてこれらの遺伝子の変異解析を開始した。今後遺伝子変異・変化が同定されれば、新規責任遺伝子となる。これまでに5つの責任遺伝子を選択しHRMにてスクリーニングを終了するも病的な遺伝子多型・変異は同定されていない。さらにスクリーニングを継続する。

V. 関連・責任遺伝子型とJIAの臨床病型の比較検討（横田・松本）

責任遺伝子が単離されたらJIAの詳細な臨床情報との比較検討を行う予定である。

本研究は**横浜市立大学倫理委員会の承認を受け適切な倫理的配慮と手続き**を経て行われた。

D．考察

全身型JIAの遺伝的背景はほとんど不明で本研究班が目指す責任・関連遺伝子の解明の学術的・臨床的意義は大きい。期待されているIL-6受容体に対するモノクローナル抗体の有効性は高いが、約15%の症例で全身炎症は沈静化しても関節破壊が進行する症例が存在し、責任・関連遺伝子を同定しその分子病態を解明していく必要がある。当初責任遺伝子の可能性が高いと考えられた遺伝子Xについては残念ながら1例にのみ認められる異常であり、その機能的な関連遺伝子Yについても変異を同定できなかったことからJIAの責任遺伝子であるとの結論には至っていない。しかし同様な炎症性疾患であるA症候群の責任遺伝子であると認識されている遺伝子Xの明らかな異常を認めたにもかかわらずA症候群を呈していない点は学術的には重要である。全ゲノムマイクロアレー解析で1例において染色体19番上に両親には認めない約77 Kbと622 Kbの不連続な*de novo*重複を認めた（論文投稿中）。この領域内には約30の遺伝子が存在し、免疫系に関与する興味深い遺伝子も多数包含され（疾患責任遺伝子と相同性のある遺伝子を含む）、HRM法を用いてこれらの遺伝子のうち5つを候補遺伝子として変異解析を行ったが変異は同定できていない。今後もさらに候補遺伝子の解析を進め遺伝子変異・変化が同定していく予定である。

E．結論

当初有力な候補遺伝子と想定された遺伝子Xの異常は最終的には解析した50例のうち1例にのみ認められる異常で全身型JIAを広く説明する責任遺伝子である可能性は低いと考えている。しかし全ゲノムアレー解析を通して新たに77 Kbと622 Kbのde novo重複を同定しこの領域中に存在する30個の遺伝子を有力な候補遺伝子と捉え解析が進行中である。

F．健康危険情報

本研究遂行上、健康危機に関わる問題は生じていないが，その可能性・危険性等について把握した際には，迅速に対応していきたい。

G．研究発表

1．論文発表

Shiihara T, Maruyama K-i, Yamada Y, Nishimura A, Matsumoto N, Kato M, Sakazume S. A case of Baraitser-Winter syndrome with unusual brain MRI findings of pachygyria, subcortical band heterotopia and periventricular heterotopias. Brain Dev 32(6), 502-505, 2010.

Saitsu H, Tohyama J, Kumada T, Egawa K, Hamada K, Okada I, Mizuguchi T, Osaka H, Miyata R, Furukawa T, Haginoya K, Hoshino H, Goto T, Hachiya Y, Yamagata T, Saitoh S, Nagai T, Nishiyama K, Nishimura A, Miyake N, Komada M, Hayashi K, Hirai S, Ogata K, Kato M, Fukuda A, Matsumoto

N. Dominant negative mutations in α-II spectrin cause early onset West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. Am J Hum Genet 86(6):881-889, 2010.

Saitsu H, Kato M, Okada I, Orii KE, Kondo N, Wada T, Hoshino H, Kubota M, Arai H, Tagawa T, Kimura S, Sudo A, Miyama S, Takami Y, Watanabe T, Nishimura A, Nishiyama K, Miyake N, Osaka H, Hayasaka K, Matsumoto N. *STXBP1* mutations in severe infantile epilepsies with suppression-burst pattern. Epilepsia 51(12): 2397-2405, 2010

Sakai H[#], *Yoshida K[#] ([#]denotes equal contribution), Shimizu Y, Morita H, Ikeda S-i, Matsumoto N. Analysis of an insertion mutation in a cohort of 93 patients with spinocerebellar ataxia type 31 (SCA31) from Nagano, Japan. Neurogenet 11(4): 409-415, 2010

Osaka H, Yamamoto R, Hamanoue H, Nezu A, Sasaki M, Saitsu H, Kurosawa K, Shimbo H, Matsumoto N, Inoue K. Disrupted SOX10 regulation of GJC2 transcription causes Pelizaeus-Merzbacher-Like Disease. Ann Neurol 68(2): 250-254, 2010

Nishimura A, Hiraki Y, Shimoda H, Tadaki H, Tsurusaki Y, Miyake N, Saitsu H, Matsumoto N. *De novo* deletion of 1q24.3-q31.2 in a patient with severe growth retardation. Am J Med Genet 152A:1322-1325, 2010

Komoike Y, Fujii K, Nishimura A, Hiraki Y, Michiko, Hayashidani M, Shimojima K, Nishizawa T, Higashi K, Yasukawa K, Saitsu H, Miyake N, Mizuguchi M, Matsumoto N, Osawa M, Kohno Y, Higashinakagawa T, Yamamoto T. Zebrafish gene knockdowns imply roles for human *YWHAG* in infantile spasms and cardiomegaly. Genesis 48(4): 233-243, 2010.

Doi H, Koyano S, Miyatake S, Matsumoto N, Kameda T, Tomita A, Miyaji Y, Suzuki Y, Sawaishi Y, Kuroiwa Y. Siblings with the adult-onset slowly progressive type of pantothenate kinase-associated neurodegeneration and a novel mutation, Ile346Ser, in PANK2: Clinical features and (99m)Tc-ECD brain perfusion SPECT findings. J Neurol Sci 290 (1-2): 172-176, 2010.

Miyake N, Kosho T, Mizumoto S, Furuichi T, Hatamochi A, Nagashima Y, Arai E, Takahashi K, Kawamura R, Wakui K, Takahashi J, Kato H, Yasui H, Ishida T, Ohashi H, Nishimura G, Shiina M, Saitsu H, Tsurusaki T, Doi H, Fukushima Y, Ikegawa S, Yamada S, Sugahara K, Matsumoto N. Loss of decorin dermatan sulfate impairing collagen bundle formation in a new type of Ehlers-Danlos syndrome. Hum Mut 31(8): 966-974, 2010

Kosho T, Miyake N, Hatamochi A, Takahashi J, Kato H, Miyahara T, Yasui H, Ishida T, Ono K, Kosuda T, Inoue A, Kohyamab M, Hattori T, Ohashi H, Nishimura G, Kawamura R, Wakui K, Fukushima Y, Matsumoto N. A New Ehlers-Danlos Syndrome With Craniofacial Characteristics, Multiple Congenital Contractures, Progressive Joint and Skin Laxity, and Multisystem Fragility-related Manifestations. Am J Med Genet 152A (6): 1333-1346, 2010.

Kimura S, Saitsu H, Blanka A, Schaumann BA, Shiota K, Matsumoto N, and Ishibashi M. Rudimentary Claws and Pigmented Nail-like Structures on the Distal Tips of the Digits of *Wnt7a* Mutant Mice: *Wnt7a* Suppresses Nail-like Structure Development in Mice. Birth Defects Res A Clin Mol Teratol 88(6):487-496, 2010

Kanazawa K, Kumada S, Mitsuhiro K, Saitsu H, Kurihara E, Matsumoto N. Choreo-ballistic movements in a case carrying a missense mutation in syntaxin binding protein 1 gene. Mov Disord 25(13):2265-2267, 2010

Ng S, Bigham A, Buckingham K, Hannibal M, McMillin M, Gildersleeve H, Beck A, Tabor H, Cooper G, Mefford H, Lee C,

Turner E, Smith J, Rieder M, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson D, Bamshad M, Shendure J. Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome. Nat Genet 42(9): 790-793, 2010.

Yamada-Okabe T, Imamura K, Kawaguchi N, Sakai H, Yamashita M, Matsumoto N. Functional characterization of the zebrafish *WHSC1*-related gene, a homologue of human *NSD2*. Biochem Biophys Res Commun 402(2): 335-339, 2010.

Okada I[#], Hamanoue H[#], ([#] denotes equal contribution) Terada K, Tohma T, Megarbane A, Chouery E, Abou-Ghoch J, Jalkh N, Cogulu O, Ozkinay F, Horie K, Takeda J, Furuichi T, Ikegawa S, Kiyomi Nishiyama K, Miyatake S, Nishimura A, Mizuguchi T, Niikawa N, Hirahara F, Kaname T, Yoshiura K-i, Tsurusaki Y, Doi H, Miyake N, Furukawa T, *Matsumoto N, *Saitsu H. *SMOC1* is essential for ocular and limb development in humans and mice. Am J Hum Genet 88(1): 30-41, 2011

Tohyama J, Kato M, N, Kawasaki S, Kawara H, Matsui T, Akasaka N, Ohashi T, Kobayashi Y, Matsumoto N. Dandy-Walker malformation associated with heterozygous *ZIC1* and *ZIC4* deletion: Report of a new patient. Am J Med Genet 155(1): 130-131, 2011

Furuichi T, Dai J, Cho T-J, Sakazume S, Ikema M, Matsui Y, Baynam G, Nagai T, Miyake N, Matsumoto N, Ohashi H, Unger S, Superti-Furga A, Kim O-H, Nishimura G, *Ikegawa S. *CANT1* is also responsible for Desbuquois dysplasia, type 2 and Kim variant. J Med Genet 48(1): 32-37, 2011

Saitsu H, Hoshino H, Kato M, Nishiyama N, Okada I, Yoneda Y, Tsurusaki Y, Doi H, Miyake N, Kubota M, Hayasaka K, Matsumoto N. Paternal mosaicism of a *STXBP1* mutation in Ohtahara syndrome. Clin Genet (in press)

Yano S, Bagheri A, Watanabe Y, Moseley K, Nishimura A, Matsumoto N, Baskin B, Ray PN. Familial Simpson-Golabi-Behmel syndrome: Studies of X-chromosome inactivation and clinical phenotypes in two female individuals with *GPC3* mutations. Clin Genet (in press)

Nishimura-Tadaki A, Wada T, Bano G, Gough K, Warner J, Kosho T, Ando N, Hamanoue H, Sakakibara H, Nishimura G, Tsurusaki Y, Doi H, Miyake N, Wakui K, Saitsu H, Fukushima Y, Hirahara F, *Matsumoto N. Breakpoint determination of X;autosome balanced translocations in four patients with premature ovarian failure. J Hum Genet (in press)

２．学会発表

第９回東北出生前医学研究会（1 月 30 日於仙台）・松本直通「疾患遺伝子研究の新潮流」（特別講演）

An International Workshop on Translational Science: Clinical Use, Efficacy and Translation of Basic Discoveries (Feb 1 at Yokohama) Naomichi Matsumoto "Whole Genome Approach to the Epilepsy-Related Gene" (invited lecture)

第 19 回群馬遺伝子診療研究会（2 月 23 日於群馬大学、前橋）・松本直通「年齢依存性てんかん性脳症の最近の話題」（特別講演）

平成 21 年度厚生労働科学研究費難治性疾患克服研究事業研究成果発表会（3 月 12 日於学術情報センター、東京）松本直通・年齢依存性てんかん性脳症の分子疫学と臨床像の解明

第 113 回日本小児科学会学術集会（4 月 23 日於岩手県民情報交流センター、盛岡）・シンポジウム「先天性疾患における最近の進歩：病態解明から遺伝子診断へ」・松本直通「染色体異常からの疾患遺伝子探索 」（シンポジスト）

European Human Genetic Conference 2010・Naomichi Matsumoto, Akira Nishimura, Yoko Hiraki. De novo deletion of 1q24.3-q31.2 in a patient with severe growth retardation. (Poster presentation) (June 12-15, 2010 at Gothenburg, Sweden)

久留米大学 Pediatric Ground Rounds・松本直通「年齢依存性てんかん性脳症の新知見」（招待講演）（久留米大学医学部、7 月 16 日）

BioJapan2010 アカデミックシーズ発表会・松本直通「デルマタン 4-O-硫酸基転移酵素-1 は新しいタイプのエーラス・ダンロス症候群を引き起こす」パシフィコ横浜、9 月 30 日）

Joint Egyptian-Japanese Scientific Workshop, "A new era of genetic diseases" (organized by Ghada M.H. Abdel Salam and Naomichi Matsumoto) (National Research Center, Cairo, Egypt, Oct 3-4, 2010) Naomichi Matsumoto "Microarray technologies: Hightways to genomic aberrations" (invited lecture)

Joint Egyptian-Japanese Scientific Workshop, "A new era of genetic diseases" (organized by Ghada M.H. Abdel Salam and Naomichi Matsumoto) (National Research Center, Cairo, Egypt, Oct 3-4, 2010) Naomichi Matsumoto "Isolation of the gene responsible for a new type of Ehlers-Danlos syndrome" (invited lecture)

Joint Egyptian-Japanese Scientific Workshop, "A new era of genetic diseases" (organized by Ghada M.H. Abdel Salam and Naomichi Matsumoto) (National Research Center, Cairo, Egypt, Oct 3-4, 2010) Naomichi Matsumoto "Haploinsufficienty of *STXBP1* causes Ohtahara syndrome" (invited lecture)

The 4th Asian Chromosome Colloquium (Beijing, China, Oct 11-14) Naomichi Matsumoto "Identification of two epilepsy-related genes from a 2.25-Mb deletion in one patient." (invited lecture)

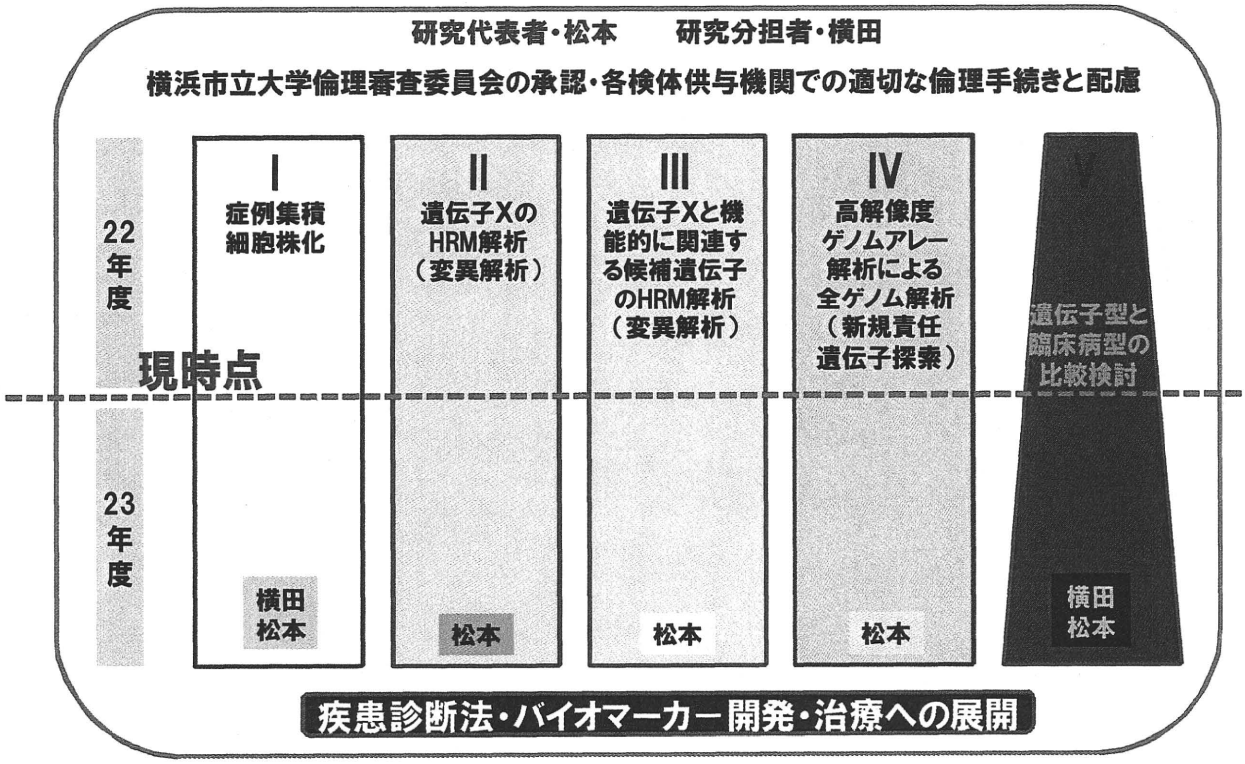
日本人類遺伝学会第 55 回大会（大宮、10 月 30 日）松本直通「疾患ゲノム解析：遺伝性疾患のエクソーム解析」（次世代シーケンサーを用いたヒト（疾患）ゲノム解析の現状セッション・シンポジスト・座長）

第 22 回 NIH 金曜会（National Institute of Health, Bethesda, MD 11 月 5 日）Naomichi Matsumoto「Identification of two genes responsible for age-dependent epileptic encephalopathy」(invited lecture)

H．知的財産権の出願・登録状況

特願 2010-106974・松本直通・Waardenburg 無眼球症候群の検出方法・横浜市立大学・平成 22 年 5 月 7 日

研究全体の流れと進行状況



平成２２年度厚生科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

若年性特発性関節炎の遺伝的要因の実態

分担研究課題： 若年性特発性関節炎発症および合併症に及ぼす遺伝要因に関する研究

分担研究者　横田俊平　横浜市立大学医学部小児科

研究要旨：
全身型若年性特発性関節炎（s-JIA）および s-JIA の重篤な合併症であるマクロファージ活性化症候群（MAS）に絡む遺伝的要因を探索した。Interferon regulatory factor 5 (IRF5)は炎症性サイトカインをコードする遺伝子群の活性化に関わるマスター転写因子でありこれまでに IRF5 多型と全身性ループス・エリテマトーデス（SLE）やリウマチ性関節炎などの自己免疫性疾患との関連が報告されてきた。81 例の s-JIA と 3 種類の IRF5 多型との関連性を調査したところ特に正常対照 190 例に比し有意な差は確認されなかった。しかし IRF5 多型の一つ rs2004640 T アリルは s-JIA の MAS 発症群に於いて非発症群と比較し高い相関を示し、IRF5 多型が s-JIA の MAS 発症に関与している可能性が示された。

A．研究目的

全身型若年性特発性関節炎（s-JIA）および s-JIA の重篤な合併症であるマクロファージ活性化症候群（MAS）に絡む遺伝的要因を明らかにすることを目的とした。Interferon regulatory factor 5 (IRF5)は炎症性サイトカインをコードする遺伝子群の活性化に関わるマスター転写因子でありこれまでに IRF5 多型と全身性ループス・エリテマトーデス（SLE）やリウマチ性関節炎などの自己免疫性疾患と関連が報告されてきた。本研究では s-JIA および MAS 合併の有無における IRF5 多型との関連を中心にその相関を明らかにすることを目的とした。

B．研究方法

研究対象は横浜市立大学病院を受診した ILAR 分類の s-JIA のクライテリアに適合した 81 例の s-JIA である。うち MAS を合併した s-JIA は 33 例、非合併の s-JIA は 48 例である。MAS の診断は臨床症状と試験的診断ガイドライン（Ravelli A et al., J Pediatr 2005）に従った。全例に於いて IRF5 の 3 つの多型（rs729302, rs2004640, rs2280714）を TaqMan SNP タイピング法を用いて解析した。解析には ABI7500 リアルタイム PCR を用いた。統計学的解析は SNPassoc パッケージを用いてロジスティック回帰分析法を用いた。

C．研究結果

s-JIA 群及び正常対照群において 3 種類の IRF5 多型の有意な頻度差は観察されなかった（いずれの多型も P=0.37~0.80 と有意で無かった）。一方 s-JIA において MAS 合併群と非合併群の比較で rs2004640 T アリルが MAS 合併群と有意に高い相関を示した。T アリルを有する群では T アリルを有さない群と比し MAS 発症リスクが Bonferroni correction 後に P=0.003, OR 4.12, 95% CI 1.84, 9.16 で、T アリルを有する群では MAS 発症リスクが有意に高かった。さらに 3 つの多型（rs729302-rs2004640-rs2280714）の A-T-T ハプロタイプは MAS 発症と有意な相関を示した（P<0.0004, OR4.61, 95% CI 1.73-12.3）。

D．考察

s-JIA において MAS は予後を左右する重要な合併症である。今回多型との関連が明らかになった IRF5 は、IL-6, IL-12, TNF-α などの炎症性サイトカインの遺伝子群の発現を誘導する。MAS においては IFN-γ や TNF-α などのサイトカインの異常が特徴であり、今回明らかにされた IRF5 との関連性は極めて興味深い結果である。

E．結論

81 例の s-JIA と 3 種類の IRF5 多型との関連性を調査したところ特に正常対照 190 例に比し有意な差は確認されなかった。しかし IRF5 多型の一つ

rs2004640 Tアリルは s-JIA の MAS 発症に於いて高い相関を示し、IRF5 が s-JIA の MAS 発症に関与している可能性が示された。s-JIA における IRF 多型の発症リスク予測等臨床上有用な指標になる可能性がある。

F．健康危険情報
特になし。

G．研究発表
1．論文発表

Yanagimachi M, Naruto T, Miyamae T, Hara T, Kikuchi M, Hara R, Imagawa T, Mori M, Sato H, Goto H, Yokota S. Association of IRF5 Polymorphisms with Susceptibility to Macrophage Activation Syndrome in Patients with Juvenile Idiopathic Arthritis. J Rheumatol. 2011 (in press)

Yanagimachi M, Miyamae T, Naruto T, Hara T, Kikuchi M, Hara R, Imagawa T, Mori M, Kaneko T, Goto H, Morita S, Mizuki N, Kimura A, Yokota S. Association of HLA-A(*)02:06 and HLA-DRB1(*)04:05 with clinical subtypes of juvenile idiopathic arthritis. J Hum Genet. 2011 (in press)

Kasai K, Mori M, Hara R, Miyamae T, Imagawa T, Yokota S. National survey of childhood febrile illness cases with fever of unknown origin in Japan. Pediatr Int. 2011 (in press).

Yanagimachi M, Naruto T, Hara T, Kikuchi M, Hara R, Miyamae T, Imagawa T, Mori M, Kaneko T, Morita S, Goto H, Yokota S. Influence of polymorphisms within the methotrexate pathway genes on the toxicity and efficacy of methotrexate in patients with juvenile idiopathic arthritis. Br J Clin Pharmacol. 2011: 71(2):237-243.

Yokota S, Kishimoto T. Tocilizumab: molecular intervention therapy in children with systemic juvenile idiopathic arthritis. Expert Rev Clin Immunol. 2010: 6(5):735-743.

Tateishi U, Imagawa T, Kanezawa N, Okabe T, Shizukuishi K, Inoue T, Yokota S. PET assessment of disease activity in children with juvenile idiopathic arthritis. Pediatr Radiol. 2010: 40(11):1781-1788

Yokota S, Mori M, Imagawa T, Murata T, Tomiita M, Itoh Y, Fujikawa S, Takei S. Guidelines on the use of etanercept for juvenile idiopathic arthritis in Japan. Pediatric Rheumatology Association of Japan. Mod Rheumatol. 2010: 20(2):107-113

横田俊平 専門医のためのアレルギー学講座 アレルギー・免疫疾患の新規治療薬と治療法 小児における生物学的製剤の使い方. アレルギー59(7) : 785-794, 2010

森雅亮、横田俊平 関節症状を伴う若年性特発性関節炎におけるメトトレキサートの適応拡大の取得. 日本小児科学会雑誌 114(3) : 415-425, 2010

横田俊平 ステロイドの使い方 小児に対するステロイドの使い方. 日本医師会雑誌 139 (3) : 650-651, 2010

横田俊平 小児難病を知る 若年性特発性関節炎. Journal of Clinical Rehabilitation 19 (5) : 475-477, 2010

横田俊平 若年性特発性関節炎とマクロファージ活性化症候群 病態の把握とストラテジー. Source : Medical Science Digest 36:745-747,

2010

横田俊平 目でみるシリーズ　若年性特発性関節炎 (JIA). Frontiers in Rheumatology & Clinical Immunology　4 : 4-6, 2010

横田俊平 【小児におけるリウマチ・免疫疾患と生物学的製剤】 生物学的製剤の臨床　わが国における臨床成績を中心にして. アレルギー・免疫 17:237-243, 2010

２．学会発表

横田俊平　進化する小児リウマチ医療. 第７回三島リウマチフォーラム　2010 年 3 月 6 日（大阪）

Shumpei Yokota　Clinical remission in children with systemic juvenile idiopathic arthritis receiving tocilizumab treatment. EULAR2010 June 16-19, 2010 (Rome, Italy)

横田俊平 Chronic Arthritis in Childhood and Molecular Intervention Therapy. 国際小児難病フォーラム 2010 年 7 月 17-18 日（東京）

H．知的財産権の出願・登録状況
なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tadaki H, et al., Yokota S, Matsumoto N.	*De novo* 19q13.42 duplications involving *NLRP* gene cluster in a patient with systemic-onset juvenile idiopathic arthritis.	J Hum Genet	印刷中		2011
Yanagimachi M, et al., Yokota S.	Association of IRF5 Polymorphisms with Susceptibility to Macrophage Activation Syndrome in Patients with Juvenile Idiopathic Arthritis.	J Rheumatol	印刷中		2011
Yanagimachi M, et al, Yokota S.	Association of HLA-A(*)02:06 and HLA-DRB1(*)04:05 with clinical subtypes of juvenile idiopathic arthritis.	J Hum Genet	印刷中		2011
Kasai K, et al., Yokota S.	National survey of childhood febrile illness cases with fever of unknown origin in Japan.	Pediatr Int	印刷中		2011
Yanagimachi M, et al., Yokota S.	Influence of polymorphisms within the methotrexate pathway genes on the toxicity and efficacy of methotrexate in patients with juvenile idiopathic arthritis.	Br J Clin Pharmacol	71 (2)	237-243	2011
Yokota S, Kishimoto T.	Tocilizumab: molecular intervention therapy in children with systemic juvenile idiopathic arthritis.	Expert Rev Clin Immunol.	6(5)	735-743	2010
Tateishi U, et al., Yokota S.	PET assessment of disease activity in children with juvenile idiopathic arthritis.	Pediatr Radiol.	40(11)	1781-1788	2010
Yokota S, et al.	Guidelines on the use of etanercept for juvenile idiopathic arthritis in Japan. Pediatric Rheumatology Association of Japan.	Mod Rheumatol	20(2)	107-113	2010

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ORIGINAL ARTICLE

De novo 19q13.42 duplications involving *NLRP* gene cluster in a patient with systemic-onset juvenile idiopathic arthritis

Hiromi Tadaki[1,2], Hirotomo Saitsu[1], Akira Nishimura-Tadaki[1], Tomoyuki Imagawa[2], Masako Kikuchi[2], Ryoki Hara[2], Utako Kaneko[2], Takayuki Kishi[2], Takako Miyamae[2], Noriko Miyake[1], Hiroshi Doi[1], Yoshinori Tsurusaki[1], Haruya Sakai[1], Shumpei Yokota[2] and Naomichi Matsumoto[1]

Systemic-onset juvenile idiopathic arthritis (s-JIA) is a rare inflammatory disease classified as a subtype of chronic childhood arthritis, manifested by spiking fever, erythematous skin rash, pericarditis and hepatosplenomegaly. The genetic background underlying s-JIA remains poorly understood. To detect disease-related copy number variations (CNVs), we performed single-nucleotide polymorphism array analysis in 50 patients with s-JIA. We detected many CNVs, but most of them were inherited from either of normal-phenotype parents. However, in one patient, we could identify two *de novo* microduplications at 19q13.42 with the size of 77 and 622 kb, separated by a 109-kb segment of normal copy number. The duplications encompass *NLRP* family (*NLRP2*, *NLRP9* and *NLRP11*) as well as *IL11* and *HSPBP1*, all of which have an important role in inflammatory pathways. These genes may be significantly contributed to the pathogenesis of s-JIA.
Journal of Human Genetics (2011) **0**, 000–000. doi:10.1038/jhg.2011.16

Keywords: arthritis; *de novo*; duplication; systemic-onset JIA

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a chronic rheumatic disease of childhood. Approximately 11% of JIA patients show systemic-onset JIA (s-JIA),[1] which is clinically manifested by spiking fever, erythematous skin rash, pericarditis and hepatosplenomegaly in addition to arthritis. Abnormal innate immunity involving cytokines such as interleukin (IL)-1 and IL-6, neutrophils and monocytes/macrophages may play a major role in the pathogenesis of s-JIA. One of the major features of s-JIA is its progression to macrophage activation syndrome. On the basis of all these evidences, it is now generally accepted that s-JIA should be classified as an autoinflammatory syndrome rather than a classical autoimmune disease.

Two genetic factors, *HLA* and *PTPN22*, have been found as JIA susceptibility genes in multiple populations.[2] For example, HLA-B27, HLA-DR1 and HLA-DR4 have been reported to increase risk for polyarticular JIA.[3] However, such associations are seen mostly in polyarticular JIA, but not in s-JIA. Other genes including *MIF*, *IL6*, *IL10* and *TNF* are reported to be associated with s-JIA in different populations and subtypes,[4–8] although these genes account for only a small part of the total genetic contribution to JIA. Therefore, the genetic background underlying the s-JIA remains mostly undetermined.

We performed genome-wide copy number variations (CNVs) analysis in s-JIA patients. Two *de novo* microduplications at 19q13.42 encompassing 77 and 622 kb were identified in one patient by single-nucleotide polymorphism (SNP) array 6.0 and confirmed by other methods. The duplications encompass *NLRP* (Nucleotide-binding oligomeriztion domain, Leucine rich Repeat and Pyrin domain) family (*NLRP2*, *NLRP9* and *NLRP11*), which have important roles in inflammatory processes as well as *IL11*, which was reported to correlate with arthritis severity in s-JIA patients. This is the first report of *de novo* CNVs in relation to s-JIA.

MATERIALS AND METHODS

Subjects

A total of 50 patients with s-JIA, which was refractory to conventional treatment and was treated with tocilizumab, were enrolled with informed consent based on the IRB-approved protocols at Yokohama City University Hospital. There were no family histories in each patient. Genomic DNA of peripheral blood leukocytes from all patients were isolated using DNA isolation systems (Quick Gene-800; Fujifilm, Tokyo, Japan).

Whole-genome SNP array and custom array analyses

To detect CNVs, two different SNP array platforms, the Genechip Human Mapping 250K array (Affymetrix, Santa Clara, CA, USA) (for 23 patients) and

[1]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan and [2]Department of Pediatrics, Yokohama City University Graduate School of Medicine, Yokohama, Japan
Correspondence: Professor N Matsumoto, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: naomat@yokohama-cu.ac.jp
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the Genome-wide Human SNP array 6.0 (Affymetrix) (for the other 27 patients), were used following the manufacturer's protocols. In brief, for the Genome-wide Human SNP array 6.0, 500-ng DNA was digested with *Nsp*I and *Sty*I (only *Nsp*I was used for 250K array). The adaptors were ligated to the digested DNA, and the ligation-mediated polymerase chain reaction (PCR) with single primer was performed. PCR products were purified by magnetic beads (Ampure; Beckman Coulter Company, Beverly, MA, USA). Microcon-YM100 (Millipore Corporation, Bedford, MA, USA) was used for purification for the 250K array. The product was fragmented, end-labeled and hybridized to an array. CNAG3.0 (ref. 9), Genotyping Console (Copy Number Analyzer for GeneChip) (Affymetrix) and Partek Genomic Suite (Partek, St Louis, MO, USA) were used to validate copy number alterations. The qualities of the results were high in every sample (250K array: SNP call rate >95%, multifactor dimensionality reduction >99%, (multifactor dimensionality reduction−minimum candidate region) <5%; SNP array 6.0: contrast quality control >2, quality control call rate >93%, MAPD <0.4).

Q1

We also performed custom high-density oligonucleotide array-comparative genomic hybridization (CGH) analysis using custom-made 4×72K array (Roche NimbleGen, Madison, WI, USA) based on the manufacturer's protocol. This slide covered the genomic region of chromosome 19 between 59 510 019 and 61 490 039 bp with 71 891 probes. The average probes spacing are 20 bp, and the probes encompassing two duplication regions (60 144 400–60 429 000 and 60 860 000–61 051 000 bp) are 10 bp. DNA (500 ng) was sonicated with the condition of 10 s, power level 1, pulse 1 s and duty 50% using SONIFIRE-250D (Branson, Danbury, CT, USA). Patient's DNA was labeled with Cy3-random nonamers and patient's parent (father or mother) was labeled Cy5-random nonamers. Dyes were swapped in the combination of father/patient and mother/patient (patient's DNA with Cy5 and parent DNA with Cy3). Labeled DNA was hybridized at 42 °C for 16–20 h, and washed. The data analysis was carried out by NimbleScan (Roche NimbleGen) and visualized by SignalMap (Roche NimbleGen).

Fluorescence *in situ* hybridization analysis

Fluorescence *in situ* hybridization (FISH) analysis was performed on metaphase chromosomes and interphase nuclei of the patient's and parental peripheral blood leukocytes. The bacterial artificial chromosome clone, RP11-384F2, mapped to the duplicated segment was used as a probe (60 374 564–60 616 811 bp at 19q13.42, UCSC Genome Browser on Human, March 2006: hg18). Bacterial artificial chromosomes were labeled with Cy3-dUTP (Invitrogen, Carlsbad, CA, USA) by Nick translation kit (Vysis, Des Plaines, IL, USA). Probe-hybridization mixtures (16 µl) were mounted on chromosomes/nuclei, incubated at 37 °C for 16–72 h and washed. Chromosomes and nuclei were counterstained with 4′,6-diamidino-2-phenylindol containing antifade solution. Fluorescence photomicroscopy was performed under an AxioCam MR CCD fitted to Axioplan2 fluorescence microscope (Carl Zeiss, Oberkochen, Germany).

Quantitative real-time PCR to confirm copy number changes

The deletion breakpoints were analyzed using genomic DNAs by quantitative real-time qPCR with Quantifast SYBR Green PCR kit on Rotor-Gene™ 6200 HRM (Corbett Life Science, Sydney, Australia). The delta-delta Ct relative quantitative method was employed according to the manufacturer's protocol. Averages of duplicates were calculated by ROTOR-GENE 6000 SERIES software (Corbett Life Science).

Real-time reverse transcription-PCR

Lymphoblastoid cell lines established from the patient and her parents, and three normal controls were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1×antibiotic–antimycotic (Invitrogen) and 8 µg ml^{-1} tylosin (Sigma) at 37 °C in a 5% CO_2 incubator. Total RNA was independently extracted twice using RNeasy Plus Mini Kit (Qiagen). Total RNA (2 µg) was subjected to reverse transcription (RT) using PrimeScript first-strand synthesis kit with random hexamers (Takara, Ohtsu, Japan). Quantitative real-time RT-PCR was performed using TaqMan Gene expression assays (Applied Biosystems) (FAM label: Hs00174148_m1 for *IL11*, Hs00215284_m1 for *NLRP2*, Hs00603423_m1 for *NLRP9*, Hs00935472_m1 for *NLRP11*; VIC-label: Human ACTB (β-actin) Endogenous Control). Multiplex quantitative real-time RT-PCR was carried out by the two standard curve methods on RoterGene-6200 HRM (Corbett Life Science). Relative gene expression was calculated in comparison with that of the reference (used in standard curve) cDNA. The data from duplicated experiments using two distinctive RNA samples were averaged and the standard deviation was calculated.

Q2

RESULTS

Clinical features of the proband with *de novo* duplications

The proband is a 15-year-old female subject who developed s-JIA at 2 years old, with swelling of her left knee and limping. She was resistant to antibiotics, anti-inflammatory therapy, methylprednisolone pulse therapy, cyclosporin A, mizoribine, methotrexate and azathioprine. As she was resistant to all the conventional therapies, she was admitted to Yokohama City University Hospital at 9 years old and received tocilizumab (anti-IL-6 receptor antibody) therapy. After starting tocilizumab, her condition got recovered and stable since then.

Whole-genome oligonucleotide SNP array

We performed analysis of 50 patients of s-JIA using whole-genome oligonucleotide SNP array (250K *Nsp*I or SNP 6.0; Affymetrix). The total copy number abnormalities were 9 deletions and 12 duplications (Table 1).

Confirmation of CNVs by FISH and qPCR

Copy number changes were confirmed by other methods like FISH or qPCR and their origin was also checked using parental DNA if available. Most of the copy number abnormalities were of either paternal or maternal origin (Table 1), but two duplications at 19q13.42 on the patient (ID1395) occurred *de novo*, being confirmed by FISH analysis (Figure 1) and qPCR (data not shown).

Custom array-CGH

In addition, we performed custom oligonucleotide 72K array-CGH (Roche NimbleGen), to check the precise size of the duplications. CGH was performed using the following combination of test DNA/reference DNA: patient/father and patient/mother. Dye-swap analysis was also performed (Figure 2). As a result, two *de novo* microduplications at 19q13.42 with the size of 77 kb (Genome browser version hg18, chromosome 19 coordinates: 60 190 370–60 267 627 bp) and 622 kb (60 377 092–60 991 185 bp), separated by a 109-kb segment of normal copy number (60 267 627–60 377 092 bp), were identified. Two sets of test DNA/reference DNA combinations consistently showed the duplications, indicating that the duplications occurred *de novo*. Parentage of the family was confirmed using microsatellite markers (data not shown). Similar duplication was never deposited to the DECIPHER database (http://decipher.sanger.ac.uk/syndrome), although only one overlapping deletion was found in a patient with autism.

Expression analysis of duplicated genes

IL11, *NLRP2*, *NLRP9* and *NLRP11* were chosen to see their expression as they were mapped to the duplication. Low expression of *IL11* and *NLRP9* in lymphoblasts hindered the proper evaluation. *NLRP2* and *NLRP11* did show variable expression patterns depending on lymphoblastoid cells; thus, it was indeed difficult to see the effect of duplication of these genes (data not shown).

DISCUSSION

We identified two *de novo* microduplications in one out of 50 s-JIA patients. The duplications contain several interesting genes, including

Table 1 CNVs found in s-JIA patients

Patient ID	*CNV overlap (%)*	*Size*	*Deletion*	*Duplication*	*Confirmation*	*Inheritance*	*Array type*
1239	Partial CNV	267 kb		dup(3)(p12.2)	qPCR	Not available	250K
1247	0	275 b	del(11)(q21)		FISH	Inherit (father)	250K
1285	0	235 kb		dup(18)(q23)	qPCR	Inherit (father)	250K
1287	Partial CNV	1.58 mb	del(2)(q13)		FISH	Inherit (mother)	250K
	0	259 kb		dup(3)(p26.1)	qPCR	Inherit (mother)	
1317	0	13 kb	del(16)(q24.1)		qPCR	Not available	SNP 6.0
1333	0	93 kb		dup(5)(q34)	qPCR	CNV	SNP 6.0
1344	36	205 kb		dup(15)(q13q15.1)	qPCR	Inherit (father)	SNP 6.0
1350	0	50 kb	del(4)(q34.2)		qPCR	Not available	SNP 6.0
1361	0	128 kb	del(2)(q13)		qPCR, FISH	Inherit (mother)	SNP 6.0
1383	0	362 kb		dup(2)(q11.2)	qPCR, FISH	Inherit (mother)	SNP 6.0
	0	558 kb		dup(2)(q11.2)	qPCR, FISH	Inherit (mother)	
1395	**27**	**622 kb**		**dup(19)(q13.42)**	**qPCR, FISH**	***De novo***	**SNP 6.0**
	58	**77 kb**		**dup(19)(q13.42)**	**qPCR, FISH**	***De novo***	
1406	0	169 kb	del(1)(q25.3)		FISH	CNV	SNP 6.0
1407	0	144 kb		dup(13)(q12.11)	qPCR	Inherit (mother)	SNP 6.0
1433	0	13 kb	del(2)(q33.1)		qPCR	Inherit (father)	SNP 6.0
1434	42	5.5 mb	del(10)(q11.21q11.23)		FISH	Inherit (mother)	SNP 6.0
1439	62	906 kb		dup(1)(q43)	qPCR	Not available	SNP 6.0
1620	0	695 kb		dup(3)(q26.31)	qPCR	Not available	SNP 6.0
1669	0	85 kb	del(16)(q24.1)		qPCR	Not available	SNP 6.0

Abbreviations: CNV, copy number variation; FISH, fluorescence *in situ* hybridization; qPCR, quantitative real-time polymerase chain reaction; s-JIA, systemic-onset juvenile idiopathic arthritis; SNP, single-nucleotide polymorphism.
CNV, overlap shows overlapping ratio of reported CNVs. Inheritance indicates parental origin of CNVs. Not available: parental samples were unavailable

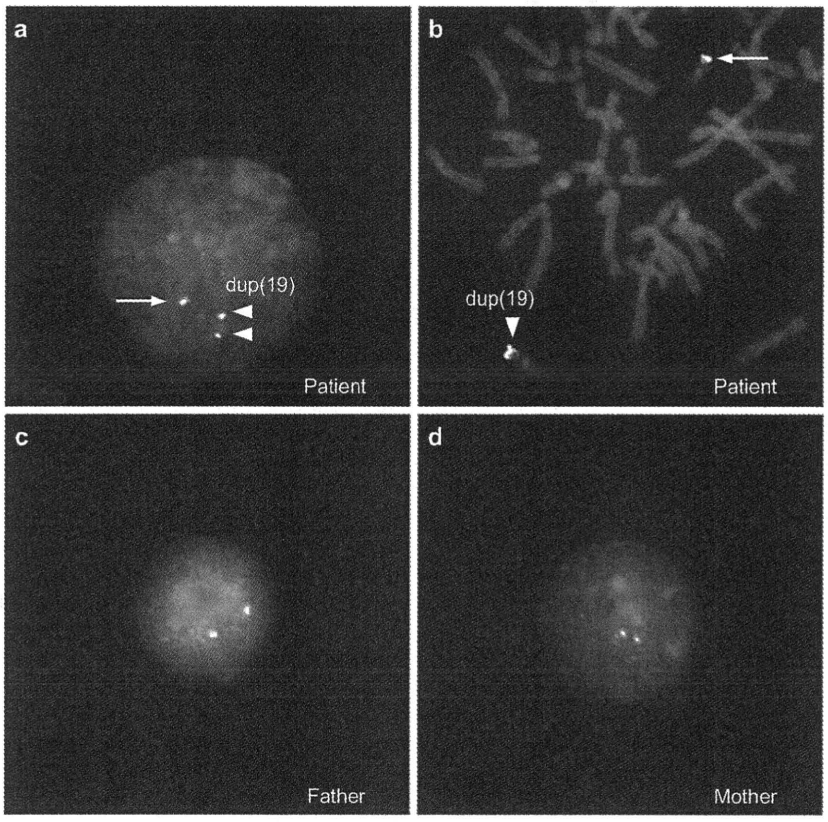

Figure 1 Fluorescence *in situ* hybridization (FISH) analysis using a bacterial artificial chromosome (BAC) clone, RP11-384F2, on cells of the family. (**a**) Interphase nucleus of the patient, (**b**) metaphase chromosomes of the patient and (**c**, **d**) father's and mother's interphase nuclei, respectively. Arrowhead indicates double signals showing duplication at 19q13.42 and arrow indicates a single signal of normal chromosome.

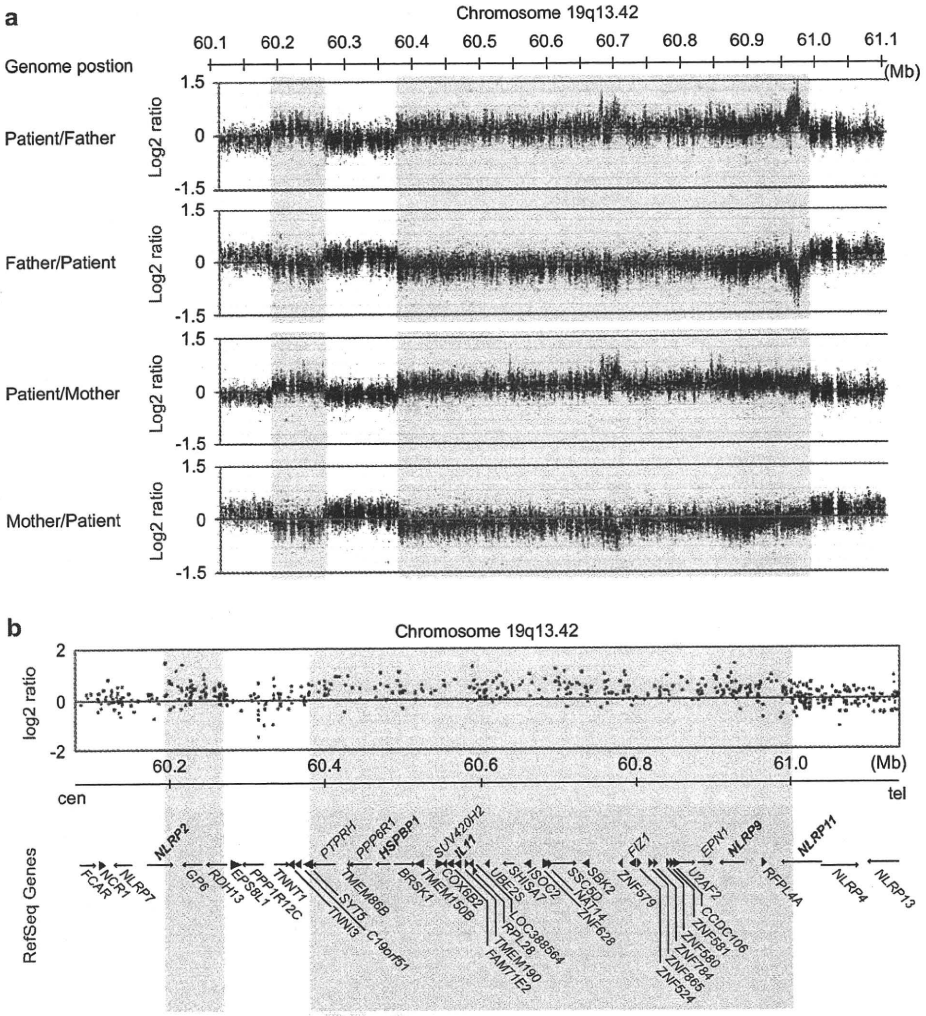

Figure 2 (**a**) Custom array-comparative genomic hybridization (CGH) results showing two *de novo* duplications at 19q13.42. Combinations of test DNA/reference DNA from top to bottom are patient/father, father/patient (dye-swapped), patient/mother and mother/patient (dye-swapped). (**b**) Characterization of 19q13.42 duplications. Upper panel shows the result of GeneChip Human SNP array 6.0 of chromosome 19 clearly showing duplications. Lower panel shows the RefSeq gene list at 19q13.42 corresponding to duplications. Duplications include *NLRP* families, *IL11* and *HSPBP1*.

NRLP family (*NLRP2*, *NLRP9* and *NLRP11*), *IL11* and *HSPBP1*, all of which are correlated with inflammatory pathways.

The *NLRP* family is composed of 14 members, including *NLRP2*, *NLRP9* and *NLRP11* (ref. 10). Most *NLRPs* are encoded by two gene clusters on chromosome 11p15 (*NLRP6*, *NLRP10* and *NLRP14*) and 19q13.4 (*NLRP2*, *NLRP4*, *NLRP5*, *NLRP7*, *NLRP8*, *NLRP9*, *NLRP11*, *NLRP12* and *NLRP13*) (ref. 10). *NLRPs* are evolutionally conserved through *Caenorhabditis elegans*, *Drosophila melanogaster*, rat, mouse and human. *NLRP1* and *NLRP3* are known as components of the inflammasome implicated in early detection of extracellular pathogens and intracellular noxious compounds and driving inflammatory and immune responses.[11] Germline mutations in *NLRP3* and *NLRP12* are associated with hereditary periodic fever syndromes.[12,13] Although the function of *NLRP9* and *NLRP11* is not well understood, *NLRP2* is suggested to function as a modulator of macrophage nuclear factor-κB activation and caspase-1 activation,[14] which may cause inflammation. All the evidences support that duplications of the *NRLP* gene cluster may significantly contribute to s-JIA pathogenesis in the patient.

IL-6 and IL-11 bind to their own ligand-specific receptor (IL-6R or IL-11R) and recruit a homodimer of gp130, which is responsible for intracellular signaling.[15] The gp130 signaling cytokines contribute to inflammation and bone homeostasis. IL-6 and IL-11 play an important role in osteoclast development, such as promoting osteoclastogenesis and bone resorption in bone marrow cultures.[16,17] IL-6 and IL-11 are capable of inducing osteoclast formation from peripheral blood mononuclear cells by a receptor activator of nuclear factor-κB ligand-independent mechanism.[18] IL-11 is produced by fibroblasts, mesenchyme-derived stromal cells of bone marrow, osteoblasts and chondrocytes.[16,19] The expression levels of IL-11 are upregulated in patients with s-JIA compared with healthy children and positively correlated with the number of joints with active arthritis.[20] Duplication of IL-11 possibly associated with its higher expression in inflammatory cells is likely to be related to the arthritis in the patient, although the roles of IL-11 are not well clarified in rheumatoid arthritis pathogenesis and alteration of IL-11 expression could not be confirmed in the patient's lymphoblastoid cells owing to the low

expression level. It is possible that the anti-IL-6 receptor antibody therapy effectively suppressed the upregulated gb130 signaling in part by IL-11 duplication in this patient.

Heat-shock proteins (Hsps) are essential to prokaryotic and eukaryotic cellular organisms during intracellular (un)folding, assembly and translocation of proteins.[21] Their synthesis is greatly enhanced in response to a variety of stressful stimuli such as temperature, hypoxia, infection and inflammation.[22] Microbial Hsps are a potential inducer of crossreactive immune responses to host self-molecules that may lead to autoimmunity.[23] In several experimental models, T cells responding to Hsps play an important role in the regulation of peripheral tolerance and suppressing pathogenic immune response.[24] HspBP1 (Hsp70 binding protein 1) has an inhibitory effect in Hsp70-assisted refolding reactions in the cytosol,[25,26] suggesting that HspBP1 plays an important role in regulating immune responses. HspBP1 duplication may alter immune responses in the s-JIA patient.

Genomic duplications can be pathogenic through both increased dosage (whole gene duplication, duplication of regulatory elements) and disruption of coding regions (intragenic duplications). Intronic duplications potentially disrupting the splicing machinery have also been reported to be pathogenic.[27] In our study, we found *de novo* duplications in one s-JIA patient, which involve many important genes for the regulation of the immune system. Other patients without any pathological CNVs in this study may have point mutations of a gene(s) mapped to the duplications, which may lead to upregulated gene expression causative for s-JIA as previously described in a different disease.[28] Further analysis is absolutely necessary.

In conclusion, this is the first report describing a possible relationship between CNVs and s-JIA, and we believe such abnormal genotypes are important to solve the pathogenesis of s-JIA.

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1 Symmons, D. P., Jones, M., Osborne, J., Sills, J., Southwood, T. R. & Woo, P. Pediatric rheumatology in the United Kingdom: data from the British Pediatric Rheumatology Group National Diagnostic Register. *J. Rheumatol.* **23,** 1975–1980 (1996).
2 Hinks, A., Barton, A., Shephard, N., Eyre, S., Bowes, J., Cargill, M. *et al.* Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. *Arthritis Rheum.* **60,** 258–263 (2009).
3 Glass, D. N. & Giannini, E. H. Juvenile rheumatoid arthritis as a complex genetic trait. *Arthritis Rheum.* **42,** 2261–2268 (1999).
4 Thomson, W. & Donn, R. Juvenile idiopathic arthritis genetics—What's new? What's next? *Arthritis Res.* **4,** 302–306 (2002).
5 Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J. S., Humphries, S. *et al.* The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J. Clin. Invest.* **102,** 1369–1376 (1998).
6 Donn, R. P., Shelley, E., Ollier, W. E. & Thomson, W. A novel 5′-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* **44,** 1782–1785 (2001).
7 Donn, R., Alourfi, Z., De Benedetti, F., Meazza, C., Zeggini, E., Lunt, M. *et al.* Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. *Arthritis Rheum.* **46,** 2402–2409 (2002).
8 De Benedetti, F., Meazza, C., Vivarelli, M., Rossi, F., Pistorio, A., Lamb, R. *et al.* Functional and prognostic relevance of the −173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* **48,** 1398–1407 (2003).
9 Nannya, Y., Sanada, M., Nakazaki, K., Hosoya, N., Wang, L., Hangaishi, A. *et al.* A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res.* **65,** 6071–6079 (2005).
10 Tschopp, J., Martinon, F. & Burns, K. NALPs: a novel protein family involved in inflammation. *Nat. Rev. Mol. Cell. Biol.* **4,** 95–104 (2003).
11 Drenth, J. P. & van der Meer, J. W. The inflammasome—a linebacker of innate defense. *N. Engl. J. Med.* **355,** 730–732 (2006).
12 Jeru, I., Duquesnoy, P., Fernandes-Alnemri, T., Cochet, E., Yu, J. W., , Lackmy-Port-Lis, M. *et al.* Mutations in NALP12 cause hereditary periodic fever syndromes. *Proc. Natl Acad. Sci. USA* **105,** 1614–1619 (2008).
13 Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A. & Kolodner, R. D. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle–Wells syndrome. *Nat. Genet.* **29,** 301–305 (2001).
14 Bruey, J. M., Bruey-Sedano, N., Newman, R., Chandler, S., Stehlik, C. & Reed, J. C. PAN1/NALP2/PYPAF2, an inducible inflammatory mediator that regulates NF-kappaB and caspase-1 activation in macrophages. *J. Biol. Chem.* **279,** 51897–51907 (2004).
15 Sims, N. A. & Walsh, N. C. GP130 cytokines and bone remodelling in health and disease. *BMB Rep.* **43,** 513–523.
16 Girasole, G., Passeri, G., Jilka, R. L. & Manolagas, S. C. Interleukin-11: a new cytokine critical for osteoclast development. *J. Clin. Invest.* **93,** 1516–1524 (1994).
17 Heymann, D. & Rousselle, A. V. gp130 cytokine family and bone cells. *Cytokine* **12,** 1455–1468 (2000).
18 Kudo, O., Sabokbar, A., Pocock, A., Itonaga, I., Fujikawa, Y. & Athanasou, N. A. Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. *Bone* **32,** 1–7 (2003).
19 Hill, P. A., Tumber, A., Papaioannou, S. & Meikle, M. C. The cellular actions of interleukin-11 on bone resorption *in vitro*. *Endocrinology* **139,** 1564–1572 (1998).
20 Mima, T., Ishikawa, S., Aoki, C., Yoshio-Hoshino, N., Adachi, Y., Imagawa, T. *et al.* Interleukin 11 and paired immunoglobulin-like type 2 receptor alpha expression correlates with the number of joints with active arthritis in systemic juvenile idiopathic arthritis. *Ann. Rheum. Dis.* **68,** 286–287 (2009).
21 Gething, M. J. & Sambrook, J. Protein folding in the cell. *Nature* **355,** 33–45 (1992).
22 Welch, W. J., Kang, H. S., Beckmann, R. P. & Mizzen, L. A. Response of mammalian cells to metabolic stress; changes in cell physiology and structure/function of stress proteins. *Curr. Top. Microbiol. Immunol.* **167,** 31–55 (1991).
23 Albani, S. & Carson, D. A. A multistep molecular mimicry hypothesis for the pathogenesis of rheumatoid arthritis. *Immunol. Today* **17,** 466–470 (1996).
24 van Eden, W., van der Zee, R., Paul, A. G., Prakken, B. J., Wendling, U., Anderton, S. M. *et al.* Do heat shock proteins control the balance of T-cell regulation in inflammatory diseases? *Immunol. Today* **19,** 303–307 (1998).
25 Shomura, Y., Dragovic, Z., Chang, H. C., Tzvetkov, N., Young, J. C., Brodsky, J. L. *et al.* Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. *Mol. Cell* **17,** 367–379 (2005).
26 Raynes, D. A. & Guerriero, V. Jr Inhibition of Hsp70 ATPase activity and protein renaturation by a novel Hsp70-binding protein. *J. Biol. Chem.* **273,** 32883–32888 (1998).
27 Bovolenta, M., Neri, M., Fini, S., Fabris, M., Trabanelli, C., Venturoli, A. *et al.* A novel custom high density-comparative genomic hybridization array detects common rearrangements as well as deep intronic mutations in dystrophinopathies. *BMC Genom.* **9,** 572 (2008).
28 Gimelli, S., Caridi, G., Beri, S., McCracken, K., Bocciardi, R., Zordan, P. *et al.* Mutations in SOX17 are associated with congenital anomalies of the kidney and the urinary tract. *Hum. Mutat.* **31,** 1352–1359.

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Association of *IRF5* Polymorphisms with Susceptibility to Macrophage Activation Syndrome in Patients with Juvenile Idiopathic Arthritis

Masakatsu Yanagimachi, Takuya Naruto, Takako Miyamae, Takuma Hara, Masako Kikuchi, Ryoki Hara, Tomoyuki Imagawa, Masaaki Mori, Hidenori Sato, Hiroaki Goto and Shumpei Yokota

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Association of *IRF5* Polymorphisms with Susceptibility to Macrophage Activation Syndrome in Patients with Juvenile Idiopathic Arthritis

MASAKATSU YANAGIMACHI, TAKUYA NARUTO, TAKAKO MIYAMAE, TAKUMA HARA, MASAKO KIKUCHI, RYOKI HARA, TOMOYUKI IMAGAWA, MASAAKI MORI, HIDENORI SATO, HIROAKI GOTO, and SHUMPEI YOKOTA

ABSTRACT. ***Objective.*** Systemic-onset juvenile idiopathic arthritis (systemic JIA) and macrophage activation syndrome (MAS), the most devastating complication of systemic JIA, are characterized by abnormal levels of proinflammatory cytokines. Interferon regulatory factor 5 (IRF5) is a member of the IRF family of transcription factors, and acts as a master transcription factor in the activation of genes encoding proinflammatory cytokines. Polymorphisms in the *IRF5* gene have been associated with susceptibility to autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. Our aim was to assess associations of *IRF5* gene polymorphisms with susceptibility to systemic JIA and MAS.

Methods. Three *IRF5* single-nucleotide polymorphisms (rs729302, rs2004640, and rs2280714) were genotyped using TaqMan assays in 81 patients with systemic JIA (33 with MAS, 48 without) and 190 controls.

Results. There were no associations of the *IRF5* gene polymorphisms or haplotypes under study with susceptibility to systemic JIA. There was a significant association of the rs2004640 T allele with MAS susceptibility (OR 4.11; 95% CI 1.84, 9.16; p = 0.001). The *IRF5* haplotype (rs729302 A, rs2004640 T, and rs2280714 T), which was reported as conferring an increased risk of SLE, was significantly associated with MAS susceptibility in patients with systemic JIA (OR 4.61; 95% CI 1.73, 12.3; p < 0.001).

Conclusion. *IRF5* gene polymorphism is a genetic factor influencing susceptibility to MAS in patients with systemic JIA, and IRF5 contributes to the pathogenesis of MAS in these patients. (J Rheumatol First Release Jan 15 2011; doi:10.3899/jrheum.100655)

Key Indexing Terms:
INTERFERON REGULATORY FACTOR 5 — POLYMORPHISMS
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Systemic-onset juvenile idiopathic arthritis (systemic JIA) is one of the most perplexing diseases in childhood, manifesting as spiking fever, rash, arthritis, pericarditis, and hepatosplenomegaly[1].

The systemic symptoms frequently recur in conjunction with exacerbation of the arthritis symptoms. Some studies have observed that abnormal expression of the proinflammatory cytokines such as interleukin 6 (IL-6) and IL-1ß was characteristic of systemic JIA[2,3].

The most devastating complication of JIA is macrophage activation syndrome (MAS), which is strongly associated with systemic JIA, but rarely with polyarthritis[4]. MAS is accompanied by serious morbidity and sometimes death. The increased levels of several proinflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and others correlate with the rapid development of clinical symptoms and the progression of abnormal laboratory measurements[4,5]. MAS closely resembles a reactive or an acquired form of familial hemophagocytic lymphohistiocytosis, considered to be caused by diminished natural killer (NK) cell function, and mutations of perforin (*PRF1*), *UNC13D*, and *STX11* genes[6]. Because patients with systemic JIA have decreased levels of perforin in NK cells and diminished NK cell function, it was recently suggested that *PRF1* mutations also play a role in the development of MAS in patients with systemic JIA[7,8,9]. Munc13-4 polymorphism was also associated with MAS in patients with JIA[10]. There

From the Department of Pediatrics, Yokohama City University Graduate School of Medicine; and the CNV Laboratory, DNA Chip Research Institute, Yokohama, Japan.

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M. Yanagimachi, MD; T. Naruto, PhD; T. Miyamae, MD, PhD; T. Hara, MD; M. Kikuchi, MD; R. Hara, MD; T. Imagawa, MD, PhD; M. Mori, MD, PhD; H. Goto, MD, PhD; S. Yokota, MD, PhD, Department of Pediatrics, Yokohama City University Graduate School of Medicine; H. Sato, MA, CNV Laboratory, DNA Chip Research Institute.

Address correspondence to Dr. M. Yanagimachi, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: m-yanagi@mvi.biglobe.ne.jp

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