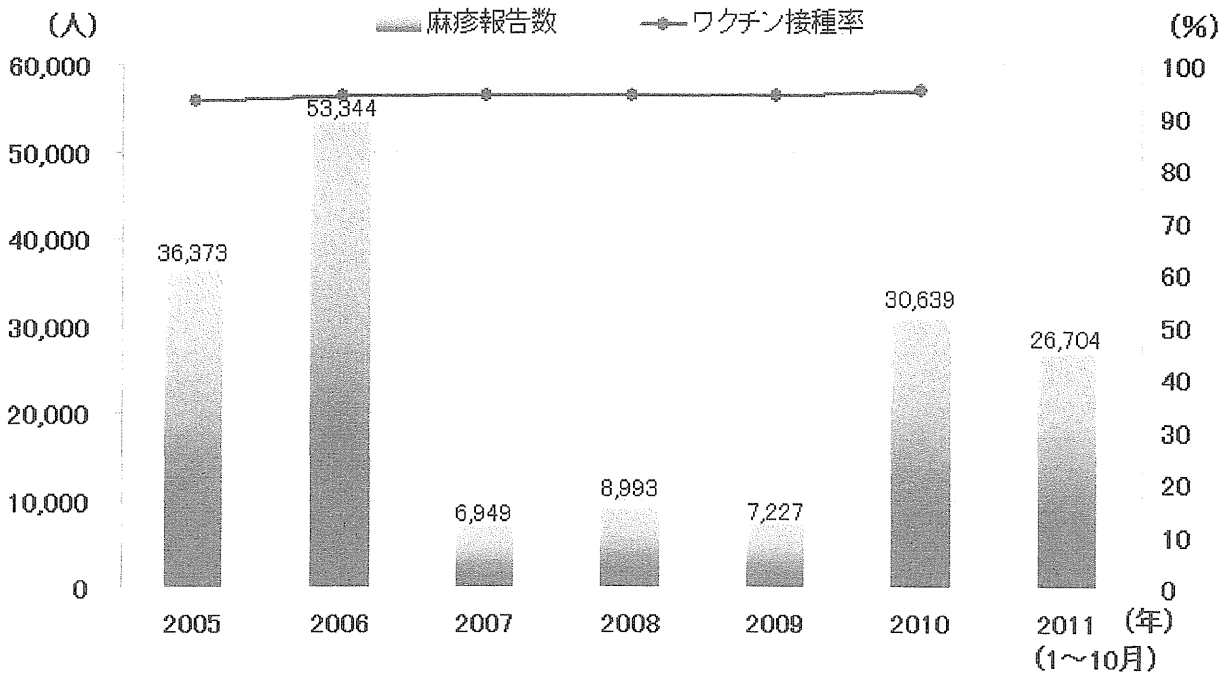


図2. EURにおける麻疹報告数の推移(2005年～2011年10月)とワクチン接種率の推移(2005年～2010年)

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# 成田空港内勤務者からのD8型麻疹ウイルスの検出と家族内感染—千葉県

(Vol. 33 p. 32-33: 2012年2月号)

2011年12月末に成田空港内勤務の患者からD8型麻疹ウイルス遺伝子を検出し、続いて2012年1月に症例の妹が発症し、同様にD8型麻疹ウイルス遺伝子を検出したので報告する。

患者は、成田空港内に勤務する22歳の女性であり、発症前1カ月間に海外渡航歴は無い。12月16日、鼻水、くしゃみ等がみられ、18日には38℃の発熱があり、19日に医療機関を受診した。初診時、麻疹は否定的であったが、いったん解熱後の20日夜に発疹が顔に出現し22日には全身に広がった。最高体温は39℃になり、その後も発熱は持続し、コプリック斑も確認されたことから24日に麻疹と診断された。患者に麻疹の既往は無く、ワクチン接種も第1期接種時に体調不良であったため接種していなかった。また、高校3年時の第4期も未接種であった。

検体は、26日に咽頭ぬぐい液、血液、尿が採取され、すべての検体から麻疹ウイルス遺伝子が検出された。

続いて発症した妹は、17歳の高校2年生で、第1期のワクチン接種済みであったが、第4期ワクチンを次年度に控え、2回目のワクチン未接種であった。1月3日に38.2℃の発熱、いったん解熱後、5日に額および腕に発疹が出現し6日に首に広がった。コプリック斑、咳、鼻水等なく軽症で経過した。

検体は、6日に咽頭ぬぐい液、血液、尿が採取され、すべての検体から麻疹ウイルス遺伝子が検出された。

検査は千葉県衛生研究所で実施した。NおよびH遺伝子に対するRT-nested PCRを実施し、検出されたN遺伝子の増幅産物について、ダイレクトシークエンスにより塩基配列を決定した。2名の塩基配列（492bp）は同一であり、決定した塩基配列の一部（456bp）について系統樹解析を実施したところ、遺伝子型D8に分類された（図1）。また、DDBJのBLAST検索の結果、MVs/Alberta.CAN/34.11/[D8]、 MVs/Dartford.GBR/4.11/[D8]等と100％の相同性を示した。

患者発生に伴う、家族および勤務先、学校に対する対応は、管轄健康福祉センターにおいて速やかに行われ、1月20日現在、2名の他に患者の発生は確認されていない。特に、妹の通う高校では、部活動で発疹出現直前に接触した4名について、接触後3日以内の緊急接種ということで、第4期の麻疹ワクチン接種の前倒しが行われ、3名が接種している。

今回の感染例は、姉については、麻疹未罹患およびワクチン未接種であったこと、成田空港という海外に開かれた場所での感染であること、妹については、高校3年時の第4期2回目のワクチン接種を次年度に控えての感染・発症というワクチン接種計画の隙間をついての患者発生であった。改めて予防接種の重要性を強調する事例であった。

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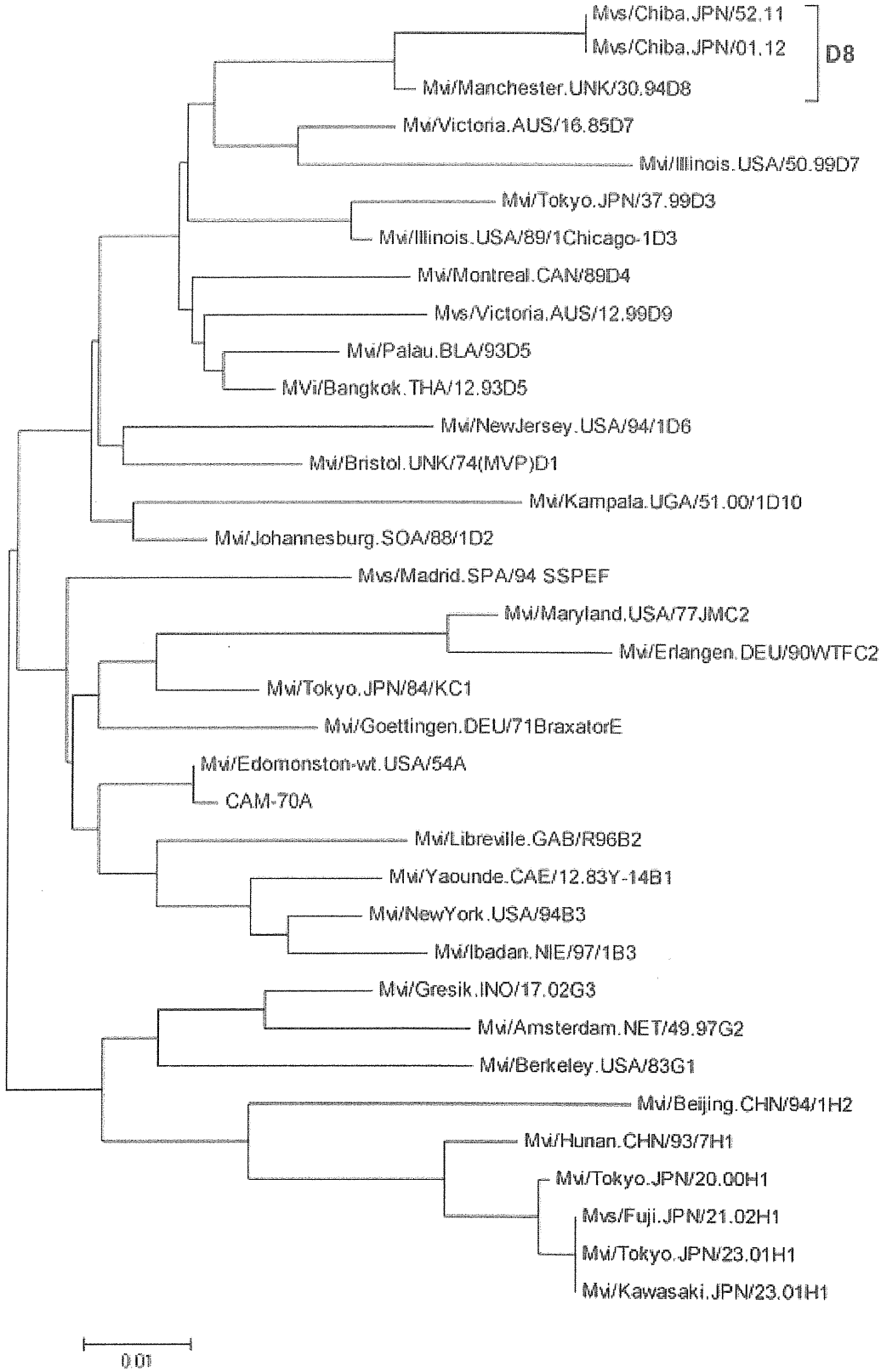


図１．麻疹ウイルスＮ遺伝子（456bp）に基づく分子系統樹

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# 地方衛生研究所の検査診断により判明したわが国の麻しんの現状

(Vol. 33 p. 41-42: 2012年2月号)

## 1．はじめに

2012年までの麻しん排除を目標にした取り組みとして、麻しん疑い症例については、全国の地方衛生研究所が全例にウイルス検査診断を行うことが、厚生労働省の通知（2010年11月）により求められている。当初、麻しん全例のPCR検査は、地方衛生研究所にとって過剰な負担ともなりかねないと危惧されたが、この２年間の麻しん疑い症例数の激減もあって、ほぼ混乱なく実施されているようである。得られた麻しんウイルス検査の結果から、わが国の麻しんの発生状況の詳細が判明したと同時に、麻しんの診断根拠および届け出に付随する新たな問題点も浮上してきている。

## 2．地方衛生研究所での麻しんウイルスの検出状況

2010年１～12月のわが国の麻しん報告数は457例であったが1)、このうち麻しんウイルスの分離・検出はわずか27例であった2)。しかしながら、2010年末に疑い症例全例でのPCR検査が勧奨された結果、2011年１～12月の麻しん報告数434例3)に対し、麻しんウイルスの分離・検出は126例と大幅に増加し2)、地方衛生研究所でのウイルス検査が麻しん診断の根拠、届出の手順としてほぼ定着したものと考えてよいだろう。問題は検出された麻しんウイルスの型別である。これまでのわが国の常在型はD5型であり、2008年以前にはこのウイルスが多数検出されていた。しかし、2009年には３件に激減し、2010年５月の１件（千葉県）の検出を最後に、この１年半の間、D5型は全く検出されていない。2011年の検出型はA型（ワクチンタイプ）、D4、D8、D9、およびG3型であり、その多くは海外からの帰国者の発症であり、明らかな輸入例である3)。また、渡航歴の無い散発例からも検出されているが、これらは輸入例からの二次感染と考えられたが、これ以上の感染拡大はみられないため、これまでのところ輸入株が常在化したことを疑わせる事例はないようである。最終的な結論を出すのは時期尚早としても、「常在型のウイルスは排除され、検出された株は輸入型とその二次感染で、輸入株の国内常在化は起こっていない」との現状分析が成り立つ蓋然性は高い。極言すれば、これはすなわち、「麻しん排除はすでに事実上達成されている」ということではないだろうか。

## 3．IgM抗体価による検査診断の問題点

麻しんの診断根拠としては、臨床所見、血清診断、ウイルス検出の３法がある。このうち汎用されているのは、血清診断の麻しん特異的IgM抗体価検査であり、健康保険でカバーされるため、年間１万数千件もの検査が民間検査機関に依頼され、しかもその陽性率は2009年のデータでは4.6％とされている。しかし、この検査は、他の感染症による偽陽性が相当数みられること、陽性と判定された例の大部分は弱陽性であり、この抗体価だけを診断の根拠とすれば、相当数の麻しんではない例の紛れ込みを許すことにつながるという問題点を抱えている。事実、数カ所の地方衛生研究所が行った偽陽性が疑われる症例での追加のウイルス検索では、風疹、ヒトパルボウイルス（伝染性紅斑）などが検出されており、全国で相当数の紛れ込み症例が麻しん報告例に実際にカウントされている可能性を強く示唆している。年間の麻しん発生数が数千以上であった2008年以前には問題にならなかった紛れ込み症例を把握することも、発生数が激減し、排除期限が目前に迫った現状では無視できない課題と考えなければならない。

## 4．麻しんの診断根拠と届出の問題点

2011年の麻しん報告例434件のうち、検査診断例は201件、臨床診断例は123件、修飾麻しん（検査診断例）が110件である3)。修飾麻しんを含めた検査診断例311件のうち、ウイルス検出は126件であり2)、残りの185件は民間検査機関の血清抗体価を診断根拠とする例数と考えられる。血清診断 185例と臨床診断123例、すなわち麻しんウイルスが証明されていない308件の麻しん報告例のうちで、いったいどれくらいの割合で実際は麻しんでないものが含まれているのか、これは推測するしかないが、半数とまではいかないにしても少なくとも20～30％にはなるのではないかと考えられる。その根拠は、適切な時期に採取された検体であるにもかかわらずウイルスが検出されず、したがって麻しんは否定的であるのに、麻しんとして届出がされている、あるいは届出が取り下げられていない事例がかなりあることが、地方衛生研究所の調査で判明しているからである。例えば、群馬県では2011年に５件の報告があるが、こ

のうち２件は群馬県衛生環境研究所での検査で、患者検体からウイルスが検出されなかったにもかかわらず、届出が取り下げられなかったものであり、麻しんでない可能性がきわめて高いと考えられる。また、残り３件は臨床あるいは血清診断で届けが出されており、ウイルス診断が行われなかったものであり、得られた情報からは、これらも麻しんの可能性は少ないと判断されるためである。

**５．麻しんの疾患様態の解明**

この１年間の地方衛生研究所が行ったウイルス検査によって、麻しんというウイルス感染症の様態についての理解が飛躍的に進んだとの見方も成り立つ。ある意味では、これは麻しんというウイルス感染症の疾患概念の変更を迫る事態であるかもしれない。ウイルス感染症の診断は歴史的に３つの段階を経て進歩した。第一の段階は、臨床症状による経験的・主観的診断である。この時代は医師が麻しんと診断すればそれだけで麻しんと認められた。第二の段階はそれに血清診断を加えて診断する段階である。血清抗体価という客観性が加わったものの、紛れ込み症例を有効に排除するまでには至らなかった。第三の段階では、直接ウイルスを検出することで確定診断ができるようになった。麻しんは不顕性感染が殆どないので、ウイルスの証明イコール確定診断である。また、適切な時期に採取された咽頭ぬぐい液・血液・尿の３種の患者検体の、いずれからもウイルスが検出されなければ、まず麻しんは否定されるものと考えてよい。この１年間、全国で多くの麻しん疑い症例でルーチンにウイルス検査が行われるようになって判明したことは、第一の段階、第二の段階での報告数には、麻しんでないものを誤認していた例数がかなり存在したにちがいないということだろう。全例にウイルス検査を行うという、手間も費用もかかるが画期的ともいえる世界に類を見ない取り組みを、今後も全国の地方衛生研究所が続けることによって、麻しんという疾患に対する理解が、さらに詳細かつ明瞭になるだろう。WHO がこの業績を正当に評価してくれることを切に望むものである。

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(掲載日 2012/2/20)

# ＜速報＞渡航歴の無い小児および家族内感染者からのD8型麻疹ウイルス検出ー愛知県

2012年2月10日までに愛知県内で麻疹と診断された患者のうち7例から、D8型麻疹ウイルス遺伝子を検出した。疫学調査では7例すべての患者および同居者に患者発症前1カ月間の渡航歴は無く、県内の2医療圏に集中している。愛知県衛生研究所で行った7例の麻疹ウイルス遺伝子検査およびウイルス分離の概要を報告する。

## 1） 医療圏Aの状況

**患者1**：11歳男児。2011年12月30日発熱。麻疹ワクチン(MCV)の接種歴無し。**患者2**：12歳女児。2012年1月2日発熱。MCV接種歴無し。患者1、2は同じ小学校の児童であるが2学期末に同小学校での有症者は確認されていない。**患者3**：患者2の兄14歳。1月10日発熱。家族内での2次感染例。MCV接種歴は不明。**患者4**：4歳女児。1月20日発熱・発疹。1回のMCV接種歴あり。患者1～3の近隣に在住するが濃厚接触を示す疫学情報は無い。他に麻疹IgM陽性を根拠に診断された1、2と同じ小学校に通う患者1名の報告あり。

## 2） 医療圏Bの状況

**患者5**：1歳男児。1月29日に発熱。MCV接種歴無し。父（1月20日発熱、麻疹IgM陽性）からの2次感染例。**患者6**：1歳男児。1月30日に発熱。MCV接種歴無し。**患者7**：6歳女児。2月3日に発熱。MCV接種歴は不明。患者５～７は発症日が近いが直接的な接触を示す疫学情報は無い。他に同医療圏より1名麻疹IgM陽性を根拠に診断された患者あり。

患者1～7より採取された血液、尿、咽頭ぬぐい液を検体として、RT-nested PCR法およびVero/hSLAM細胞を用いたウイルス分離による実験室診断を試みた。PCRの結果、ワクチン接種者以外の患者検体からは1検体を除き搬入された検体すべてで麻疹ウイルスNおよびH遺伝子が増幅され、N遺伝子の増幅産物について塩基配列を決定した。患者由来N遺伝子の部分塩基配列（456bp）はすべて同一であり、系統樹解析の結果、D8型麻疹ウイルスに分類された（図）。この部分塩基配列は千葉県が成田空港内勤者から検出を報告した配列と100%の相同性を示した（図、文献1）。また、患者１、２、５、７由来検体より各々麻疹ウイルスが分離された（中和抗体）。2月14日現在、Ａ医療圏においては他の患者の発生は確認されていないが、Ｂ医療圏においては患者発生報告が続いている。

愛知県では、2010年以降毎年輸入麻疹関連症例への対応がなされており、適切な時期に採取された検体が増えて遺伝子検出やウイルス分離率が向上している。今回報告した患者7名中6名はMCV接種歴無しまたは不明で、MRワクチン接種者は４歳（１回）児１名のみであった。ひとたび麻疹が発生するとMCV未接種者間で感染拡大がみられる（文献2, 3）ことが再認識された。日本における常在型麻疹ウイルス遺伝子検出は2010年5月を最後に報告がなく、現状では輸入関連麻疹が主体と考えられる。感染経路の特定に有用な分子疫学的解析の重要性が今後ますます高まると思われる。

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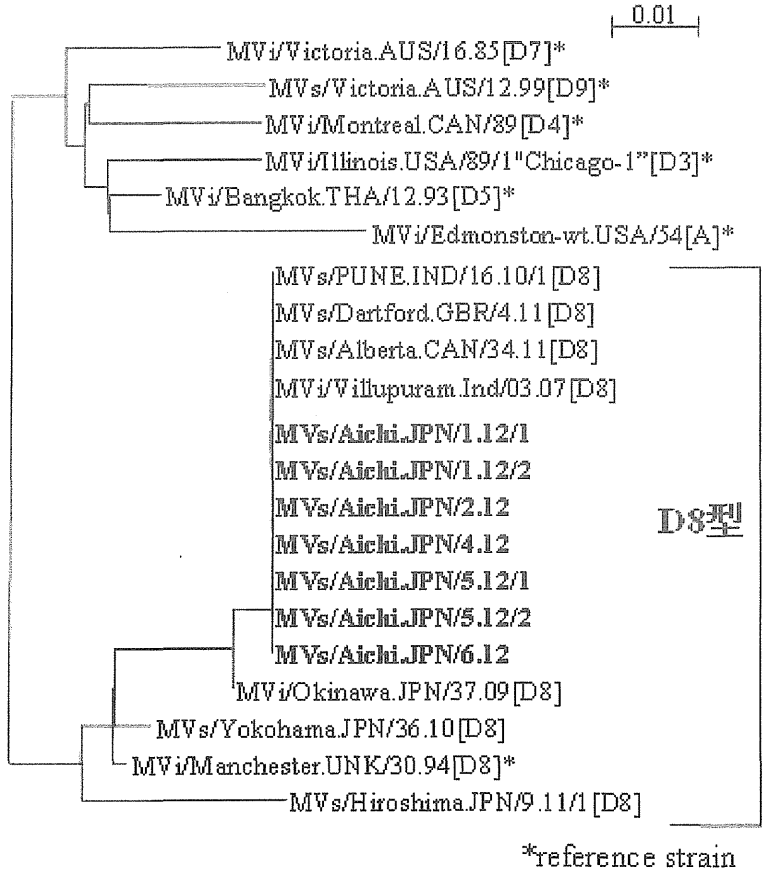


図. 麻疹ウイルスN遺伝子(456bp)の配列に基づく分子系統樹

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# 麻疹疑い症例からの風疹ウイルス検出と遺伝子型解析－愛知県

(IASR Vol. 33 p. 167-168: 2012年6月号)

愛知県においては、2011（平成23）年度より麻疹疑い検体のうち麻疹ウイルス（MeV）PCRが陰性であったすべての検体について、パルボウイルスB19および風疹ウイルスPCR検査を行っている。MeV陰性例より風疹ウイルスを検出し、遺伝子型別を行ったので報告する。

**症例１**：麻疹疑い37歳男性、2011年６月18日発病（38.8°Cの発熱、結膜炎、紅斑、バラ疹）、22日検体採取（咽頭ぬぐい液、尿、全血）。ワクチン接種歴は麻疹無し、風疹は不明。発症前１カ月以内の海外渡航歴は無い。

**症例２**：麻疹疑い38歳男性、2012年１月23日発病（38°C台の発熱、発疹、コプリック斑）、26日検体採取（尿、全血）。麻疹および風疹ワクチン接種歴は不明。発症前１カ月以内の海外渡航歴は無い。

症例１、２ともに麻疹遺伝子検査は陰性であったため、国立感染症研究所（感染研）・病原体検出マニュアルのRT-nested PCR法（プライマーA～D；方法１）1) もしくは感染研より推奨されたNS遺伝子を検出するRT-nested PCR法（方法２）により、風疹ウイルス遺伝子の検出を試みた結果、症例１の咽頭ぬぐい液検体から方法１によりE1遺伝子を、症例２の尿検体から方法２によりNS遺伝子を検出した（方法１では不検出）。これらの検体RNAよりE1遺伝子の型別領域を増幅して遺伝子型別を試みた。感染研より推奨されているOne-Step RT-PCR法によってE1内２領域の増幅を試みたが、PCR産物は得られなかった（図1A）ため、ランダムプライマーを用いた逆転写反応（麻疹検査マニュアル第２版と同じ）に引き続いて、nested PCR（各30サイクル）を行ったところ、上記E1領域が増幅された（図1B）。増幅されたE1遺伝子領域739bpを用いた遺伝子型別解析の結果、症例１は1E型、症例２は2B型に型別された（図２）。いずれの遺伝子型も中国や東南アジアを中心に世界で流行の報告があり、2010年以来日本からも報告がある2)。

わが国における麻疹の遺伝子型は、2010年５月を最後に常在型は姿を消し、本県3-5) を含む各地で輸入例からの感染拡大を示唆する結果となっている。今後は、風疹においても麻疹同様に遺伝子型別結果に基づいて輸入関連症例か否かを知る必要があるため、E1遺伝子領域の検出および遺伝子型解析は不可欠である。しかし、PCR陽性（NSおよびE1）検体からウイルスの遺伝子型が決定できたのは２割程度という報告もある6)。ウイルス量が少ない検体の場合、E1領域の検出において逆転写反応にランダムプライマーを用いることも選択肢の１つになると思われる。

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6) IASR 32: 255-257, 2011

愛知県衛生研究所
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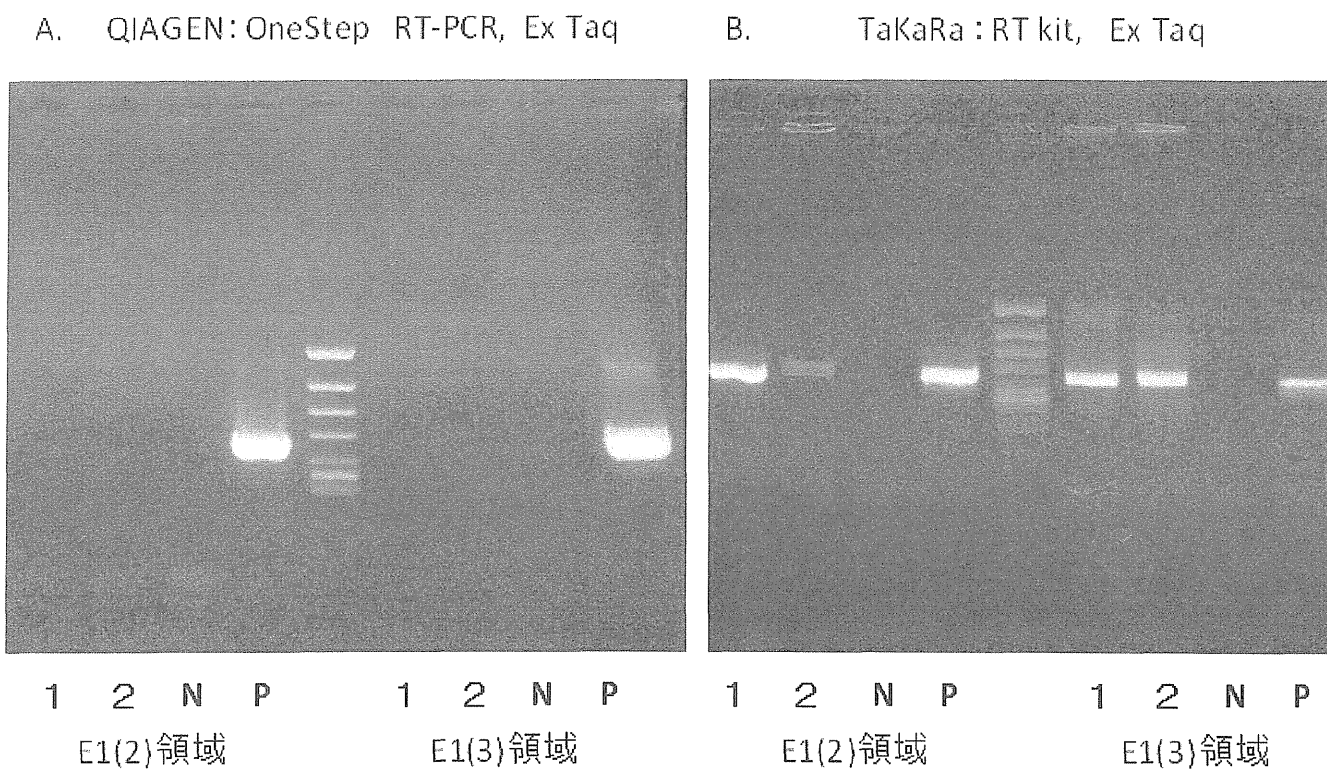
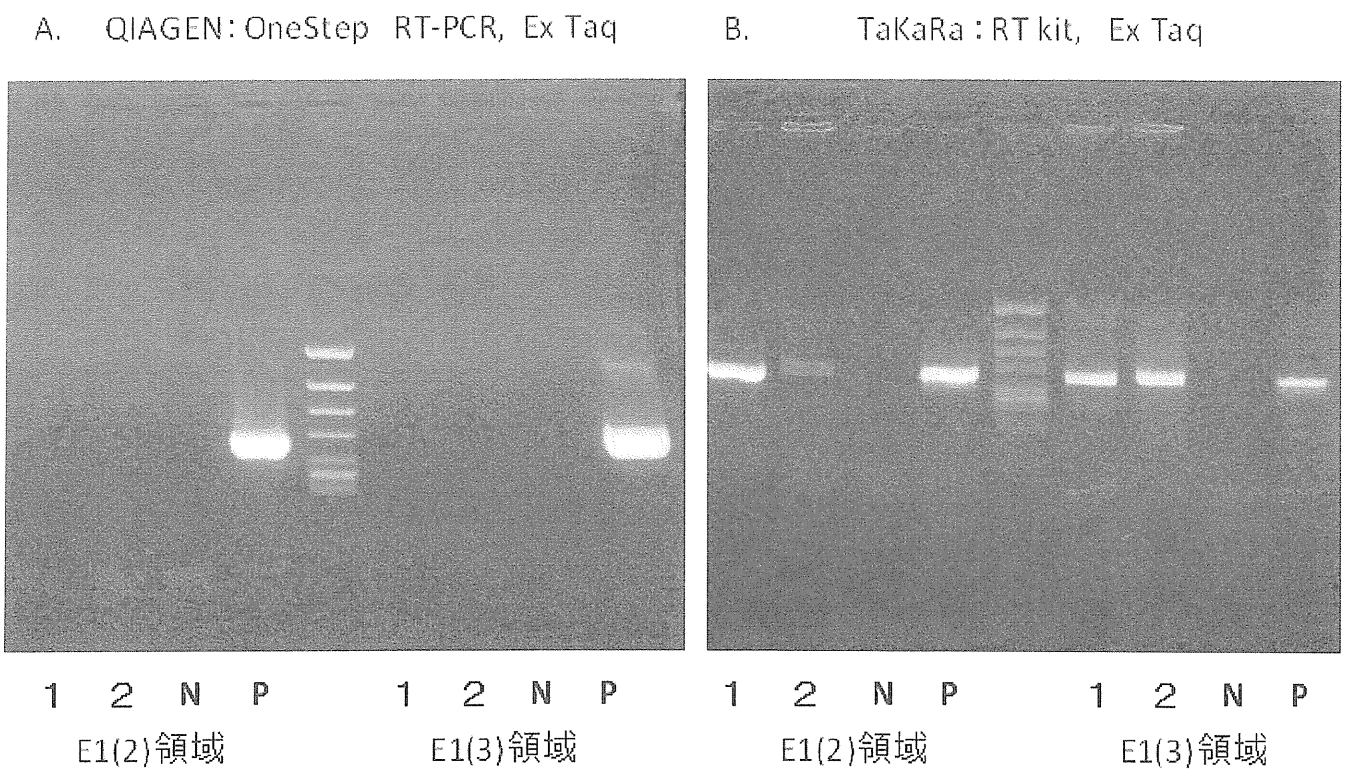



1．症例1：咽頭ぬぐい液検体
2．症例2：尿検体

**図1．臨床検体からの風疹ウイルス遺伝子E1領域の増幅**

IASR
Infectious Agents Surveillance Report

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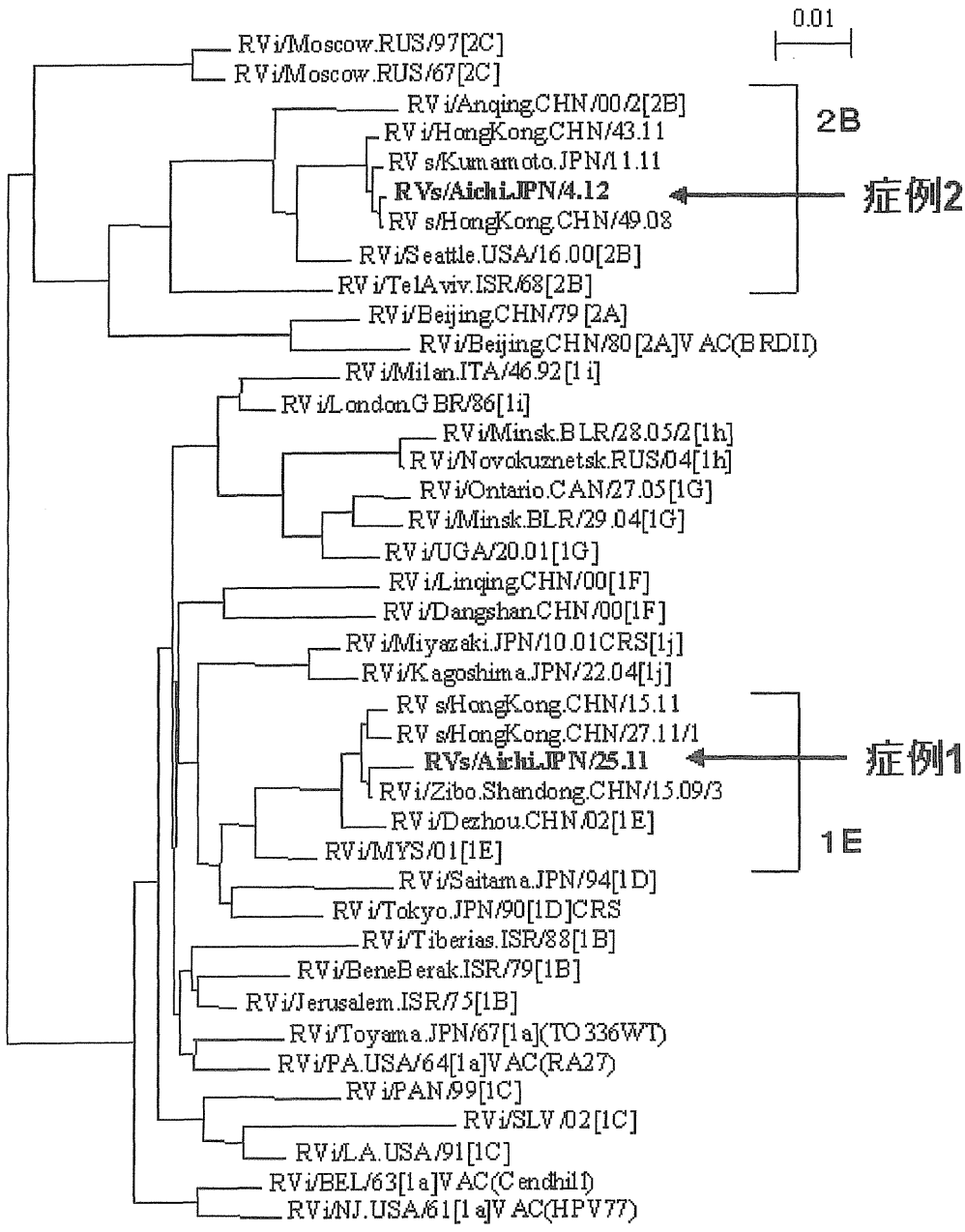


図2. 風疹ウイルスE1遺伝子（739bp）の系統樹解析

IASR

*Infectious Agents Surveillance Report*

# 研究成果の刊行物・別刷

## 2011年

# Genetic Characterization of Measles Vaccine Strains

**Bettina Bankamp,[1] Makoto Takeda,[2] Yan Zhang,[3] Wenbo Xu,[3] and Paul A. Rota[1]**

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**The complete genomic sequences of 9 measles vaccine strains were compared with the sequence of the Edmonston wild-type virus. AIK-C, Moraten, Rubeovax, Schwarz, and Zagreb are vaccine strains of the Edmonston lineage, whereas CAM-70, Changchun-47, Leningrad-4 and Shanghai-191 were derived from 4 different wild-type isolates. Nucleotide substitutions were found in the noncoding regions of the genomes as well as in all coding regions, leading to deduced amino acid substitutions in all 8 viral proteins. Although the precise mechanisms involved in the attenuation of individual measles vaccines remain to be elucidated, in vitro assays of viral protein functions and recombinant viruses with defined genetic modifications have been used to characterize the differences between vaccine and wild-type strains. Although almost every protein contributes to an attenuated phenotype, substitutions affecting host cell tropism, virus assembly, and the ability to inhibit cellular antiviral defense mechanisms play an especially important role in attenuation.**

Measles is a highly contagious disease characterized by fever, malaise, coryza, conjunctivitis, cough, and an erythematous maculopapular rash [1]. Although most patients recover from the illness, serious complications can occur, including pneumonia and invasion of the central nervous system [2, 3]. One severe complication is subacute sclerosing panencephalitis (SSPE), a rare, fatal consequence of central nervous system infection [4]. Measles caused an estimated 164,000 deaths worldwide in 2008 [5]. This number represents a 97% decrease from the estimated 6 million deaths occurring annually before the introduction of measles vaccines [6].

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Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Measles virus (MeV) was first isolated from a child with measles in a primary culture of human kidney cells [7]. This isolate, the prototype Edmonston strain, was subsequently adapted to various types of cultured cells, giving rise to several of the currently used highly attenuated, live measles vaccines [8]. The first live, attenuated vaccine, Edmonston B, was licensed in the United States under the trade name Rubeovax in 1963 [9]. It was eventually replaced by the more attenuated Moraten strain [10]. On the basis of phylogenetic analyses, MeV isolates can be assigned to 1 of 23 recognized genotypes and 1 provisional genotype [11, 12]. All vaccine strains are grouped in genotype A, possibly because genotype A was the most widely distributed genotype at the time of isolation of the vaccines' wild-type progenitors [13].

## MOLECULAR BIOLOGY OF MeV

MeV, an enveloped virus with a single-stranded, negative sense RNA genome, is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* (for review see [2]). The genome is a 15,894-nucleotide, nonsegmented RNA molecule of negative polarity. It contains 6 transcription units which are separated by nontranscribed intergenic sequences consisting of 3 nucleotides. The 6 genes (Figure 1) encode 8 proteins. In addition to the 6

structural proteins described in Figure 1, 2 nonstructural proteins are encoded by the gene for the phosphoprotein (P). The C protein is translated from an overlapping reading frame in the P gene [14], whereas the V protein is the product of an edited mRNA transcribed from the P gene [15]. The edited message has a nontemplated G residue inserted at genomic nucleotide 2499. As a result, P and V share an amino-terminal domain of 231 amino acids but have unique carboxyl terminal domains of 276 and 69 amino acids, respectively.

### The Functions of the MeV Proteins

The most abundant structural polypeptide, the nucleoprotein (N; 525 amino acids), is essential for packaging the genome into a ribonucleoprotein complex (RNP) that serves as a template for transcription, replication and is packaged into progeny virions [2]. The amino-terminal region of the N protein ($N_{CORE}$; amino acids 1–400) forms the core of the helical nucleocapsid, whereas the carboxyl-terminal region ($N_{TAIL}$; amino acids 401–525) constitutes a disordered domain located outside of the core [16]. This region is hypervariable among wild-type viruses, and the nucleotide sequence of this region is used for genotype assignation [17, 18]. The $N_{TAIL}$ region interacts with the P protein [19, 20] and the M protein [21]. Two leucine residues at amino acid positions 523 and 524 are required for the interaction with the M protein [21]. The N protein also interacts with multiple cellular proteins [22–25]. The major inducible 70-kDa heat shock protein, hsp72, promotes MeV RNA synthesis and virus propagation by interacting with the $N_{TAIL}$ region [26–28]. An asparagine residue at amino acid position 522 (Asn522) in the $N_{TAIL}$ region is important for the interaction with hsp72 [29].

The phosphoprotein (P; 507 amino acids) is a subunit of the viral RNA polymerase and acts as a chaperone that interacts with and regulates the cellular localization of N protein and probably assists in nucleocapsid assembly [20, 30, 31]. The P protein forms oligomers and binds the N protein and the polymerase protein. A domain in the amino-terminus of P is required for interactions with soluble N protein, whereas the unique carboxyl-terminus contains the self-assembly domain and binding sites for the nucleocapsid and the polymerase [2, 20, 30, 32]. The P, V (299 amino acids), and C (186 amino acids) proteins all play roles in antagonizing the host interferon (IFN) responses [33]. The V protein blocks the Jak/STAT signaling pathway and counteracts cellular IFN signaling [34–39]. It also blocks the MDA5-mediated IFN induction pathway [40–42]. Direct interference with IFN signaling by the P and C proteins has also been reported [36, 43, 44]. The C protein controls the levels of viral RNA synthesis [45, 46] to circumvent IFN induction [35, 42, 47]. The V protein binds RNA and can also regulate viral RNA synthesis [48, 49].

The matrix protein (M; 335 amino acids) lines the inner surface of the viral envelope and participates in virion maturation [2, 50]. It plays a crucial role in virus assembly by interacting with the cytoplasmic tails of the H and F proteins [51–53]. It binds the nucleocapsid and negatively regulates transcription, presumably by sequestering nucleocapsids to the plasma membrane [21, 54, 55].

The fusion protein (F) is translated as a precursor of 550 amino acids that is cleaved by cellular furin-like proteases into disulfide-linked F1 and F2 proteins. It is a cell surface–expressed, type I glycoprotein that mediates fusion with the host cell at neutral pH [2, 56].Three N-linked glycosylation sites are located in the F2 protein at amino acids 29, 61, and 67 [57]. Amino acids 113–145, located at the new amino-terminus of F1, form the hydrophobic fusion peptide [58]. This domain inserts into the membrane of the host cell during the fusion process [59]. A cytoplasmic tail of 33 amino acids at the carboxyl terminus of

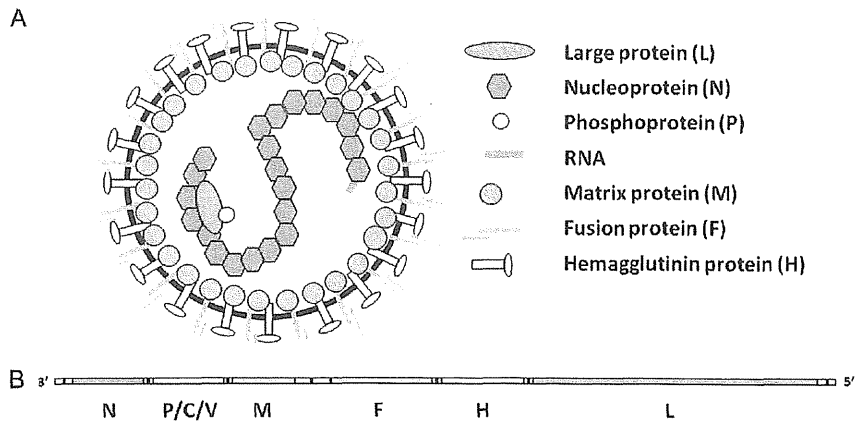


**Figure 1.** Measles virion and genome. *A*, Schematic representation of a measles virion. *B*, Organization of the measles genome. Colored rectangles represent coding regions, and white rectangles represent noncoding regions. Vertical black bars represent intergenic trinucleotides. The size of the individual regions is not to scale.

F1 interacts with the M protein and contains sorting signals for intracellular localization [60].

The hemagglutinin (H; 617 amino acids) protein is the second glycosylated surface-expressed protein. This type 2 glycoprotein mediates attachment to specific protein receptors on the host cell and is a required co-factor for fusion [2]. There are 4 N-linked glycosylation sites between amino acids 168 and 200 [61], and some wild-type isolates are additionally glycosylated at amino acid 416 [62]. A cytoplasmic tail of 34 amino acids at the amino-terminus interacts with the M protein and contains sorting signals for intracellular localization [63, 64]. Two receptors have been identified for MeV [65]. hSLAM (human signaling lymphocyte activation molecule, CD150) is a common receptor for all MeV strains [66], whereas CD46 (membrane cofactor protein, MCP) functions as a receptor for vaccines and some laboratory-adapted strains, but not for wild-type strains [67, 68]. hSLAM is only expressed on a subset of immune cells [69–71]. All vaccine strains have acquired the ability to use CD46 as an alternative receptor during passage in various SLAM-negative cultured cells [65]. The ubiquitous expression of CD46 explains the ability of vaccine strains to infect various human and monkey cell lines. Recent studies have revealed that another, presently unidentified receptor exists for wild-type MeV on epithelial cells that form tight junctions (polarized epithelial cells) [72–76], and this as-yet uncharacterized receptor is referred to as "EpiR" in this review. The H protein is the major target for neutralizing antibodies induced by vaccination [77].

The Large gene encodes the multifunctional catalytic subunit of the RNA dependent RNA polymerase (L; 2183 amino acids), which is responsible for both transcription and replication [2, 31]. It is thought to carry out most (if not all) enzymatic activities required for transcription and replication, including nucleotide polymerization, mRNA capping, and polyadenylation [78–80]. On the basis of amino acid sequence conservation among nonsegmented negative strand viruses, 6 domains of higher conservation have been characterized in the L protein [81]. Within these domains, several functional motifs have been identified. The conserved domains may represent concatenated functional domains that are connected by less-conserved hinge regions [81]. L forms oligomers and binds its co-factor, the P protein. Binding sites for these interactions have been identified [82, 83]. Together with the genome, the N, P, and L proteins constitute the transcriptionally active RNP.

### The Roles of Noncoding Regions

Nearly 11% of the 16-kb MeV genome is composed of noncoding RNA (Figure 1). The 6 transcription units are preceded by a 3′ leader and are followed by a 5′ trailer. All cis-acting regulatory elements such as promoters and encapsidation signals are contained within the first 107 and the last 109 nucleotides of the genome [84, 85]. Each gene contains 5′ and 3′ noncoding regions with conserved start and end signals for transcription and polyadenylation [86]. Morbillivirus genomes are unique within the *Paramyxoviridae* in that they contain a very long noncoding region of ~1000 nucleotides between the M and F open-reading frame (ORF) [56, 87]. The region is GC rich and is likely to fold into complex secondary RNA structures. The function of this region is not well understood, but it may play a role in regulating transcription of the M and F gene [88–90].

### Measles Virus Replication

MeV attaches to the host cell through the interaction of the viral H protein with a cellular receptor that may be hSLAM, CD46 or the as-yet unidentified EpiR [65]. After the F protein mediates fusion of the virion with the cell membrane, the negative-stranded RNP is introduced into the cytoplasm, where it acts as a template for both primary transcription of mRNAs and replication into positive-stranded antigenomic RNA (for a review, see Lamb and Parks [91]). The polymerase complex, consisting of L and P proteins, is delivered with the RNP. mRNA transcripts are capped, polyadenylated and terminated at the gene end signals [92]. The full-length positive sense antigenomic intermediate acts as a template for the replication of full-length, negative sense genomic RNA. During replication the gene end, intergenic trinucleotide and gene start signals are ignored, and both nascent positive and negative sense genomes are concomitantly encapsidated to produce RNPs [93]. F and H proteins insert into the plasma membrane, whereas M proteins line the inside of the membrane and interact with the cytoplasmic tails of the glycoproteins and the RNP [50, 52]. The virion buds from the plasma membrane to complete the replication cycle.

## ORIGINS AND PASSAGE HISTORIES OF VACCINE STRAINS

The first complete genomic sequence of a live, attenuated measles vaccine strain was published 1993 for the strain AIK-C [94] and was later updated by Parks et al [95, 96]. Since then, the genomic sequences of 8 additional vaccine strains have become available from GenBank (for accession numbers, see Figure 3). Sequence data were reanalyzed for this review; however, many of the findings were previously published [95–98]. Figure 2 summarizes the passage histories of the measles vaccines. Several of these strains were developed from the Edmonston isolate [7], whereas others were derived from wild-type progenitors isolated independently in Russia (Leningrad-4), Japan (CAM-70), and China (Shanghai-191) [99–101]. Unfortunately, the original Edmonston isolate is no longer available for analysis. Instead, vaccine sequences are compared with the Edmonston wild-type strain, which has undergone 13 passages in cell culture [8]. The AIK-C strain is used in Japan [102], where it was developed from the Edmonston strain [103]. The Zagreb strain (also referred to

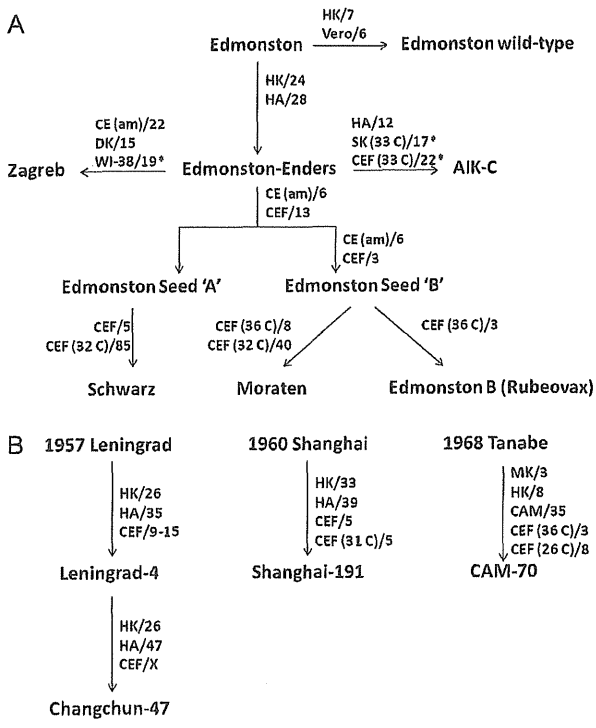


**Figure 2.** Passage histories (adapted from [8], with permission). *A*, Passage histories of Edmonston-derived vaccines. *B*, Passage histories of non–Edmonston-derived vaccines. Temperature of passages was assumed to be 37°C unless otherwise stated. A forward slash followed by a number indicates the number of passages. CAM, chorioallantoic cavity of chick embryo; CE(am), intraamniotic cavity of chick embryo; CEF, chick embryo fibroblast; DK, dog kidney; HA, human amnion; HK, human kidney; SK, sheep kidney; WI-38, human diploid cells; *Plaque purification.

as Edmonston-Zagreb) was derived from the Edmonston strain by further passaging and plaque purification in human diploid cells [104, 105]. It is produced by the Serum Institute of India and is the most commonly used measles vaccine in the

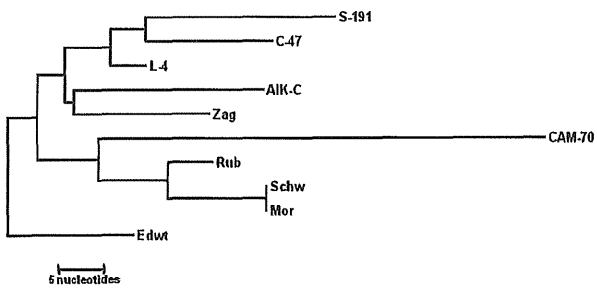


**Figure 3.** Phylogenetic tree based on complete genomic sequence for all vaccine strains. GenBank accession numbers are as follows: AF266288 (Edmonston wild-type, Edwt), AF266290 (Zagreb, Zag), AF266286 (AIK-C), AF266291 (Schwarz, Sch), AF266287 (Moraten, Mor), AF266289 (Rubeovax, Rub), FJ416068 (Changchun-47, C - 47), FJ416067 (Shanghai-191, S - 191), and DQ345723 (CAM-70). The tree was generated with MEGA4 [106] using maximum parsimony.

Expanded Programme on Immunization of the World Health Organization [107]. Edmonston B (Rubeovax) was developed from the Edmonston isolate by passaging in primary human and avian cell culture systems [108–111]. Although the vaccine induced excellent seroconversion rates, it also induced fever in 46% of vaccinees [9] and was replaced with further attenuated strains [9]. The Schwarz vaccine strain was generated in 1962 by additional passaging of the Edmonston strain in chick embryo fibroblasts [112]. The strain is produced by manufacturers in Europe and Brazil [113]. Moraten was also generated by additional passaging of the attenuated Edmonston-Enders virus in chick embryo fibroblasts [9] and it is used in the United States as Attenuvax, Merck & Co., [113]. Leningrad-4 was derived from an isolate obtained in 1957 in St Petersburg (formerly Leningrad), Russia [99]. Like Edmonston B, it proved to be insufficiently attenuated [114]. Further passaging led to the development of the Chinese vaccine strain Changchun-47 [115]. Shanghai-191 was developed from an isolate obtained in Shanghai in 1960 and attenuated through passages in human and avian cell culture systems [101]. It has been used continually since 1966 [98]. CAM-70 was derived from the Japanese wild-type isolate, Tanabe, by serial passaging in the chorioallantoic membrane of chickens [100], followed by passaging in chick embryo fibroblasts [116, 117]. CAM-70 is produced in Japan and Indonesia [113].

The first study comparing the full-length genomic sequences of the Edmonston-derived vaccine strains AIK-C, Moraten, Rubeovax, Schwarz, and Zagreb with the Edmonston wild-type strain was published in 2001 [95, 96]. It was followed by the sequences of independently derived CAM-70, Shanghai-191, and Changchun-47 [97, 98]. The sequence of Leningrad-4 was submitted to GenBank in 2004 by D. Zhao but was not published. Although the propagation of the Tanabe progenitor strain and the sequence of its H gene were reported recently [118], the remainder of the sequence of the genome of the Tanabe strain remains unavailable for comparison. The sequences of several vaccine strains that are still in use have not been determined yet. Among these are Leningrad-16, a vaccine strain derived from a Russian isolate [119, 120]; Schwarz F88, a Japanese vaccine derived by further passaging of the Schwarz strain [113]; and TD97, another Japanese vaccine that was generated from the Tanabe isolate [113].

## COMPARATIVE SEQUENCE ANALYSIS

### Phylogenetic Analysis

The total number of nucleotide changes compared with Edmonston wild-type is 29 for Leningrad-4; 33 for Zagreb; 36 for Rubeovax; 42 for AIK-C, Moraten, and Schwarz; 43 for Changchun-47; 50 for Shanghai-191; and 73 for CAM-70. Of the 3 viruses with the smallest number of substitutions, 2 (Leningrad-4 and Rubeovax) were insufficiently attenuated.

Clearly, the absolute number of substitutions alone does not signify attenuation, because the fully attenuated Zagreb strain has fewer substitutions, compared with Edmonston wild-type than Rubeovax. Figure 3 demonstrates that the 2 most divergent strains are Shanghai-191 and CAM-70 (89 nucleotide differences from each other or 0.6% of the genome), which were derived from unique wild-type progenitors. These 2 strains also have the largest number of nucleotide differences from Edmonston wild-type. This degree of divergence is likely due to their generation from non-Edmonston progenitors; however, this interpretation cannot be tested until the sequences of their progenitors become available. The most closely related strains are Rubeovax and Moraten or Rubeovax and Schwarz (16 nucleotide differences, respectively). A surprising finding was that Moraten and Schwarz have identical nucleotide sequences, despite their divergent passage histories [95, 96]. Because both viruses have been passaged in CEF at reduced temperatures (Figure 2), it is possible that similar cell culture conditions may have resulted in similar nucleotide substitutions. Despite the diverse geographic origins of the progenitors and the variations in cell culture systems, incubation temperatures, and passage numbers, the genomes of vaccines demonstrate remarkable sequence similarity. Phylogenetic analysis (Figure 3) indicated that Changchun-47 could have been derived from Leningrad-4, which is consistent with the strain's reported passage history [115]. The increased number of nucleotide substitutions in CAM-70 is distributed throughout the coding sequences of the genome rather than the noncoding sequences (Figure 4). All genes—but especially the P/C/V gene— are affected, whereas the variability of the M gene is similar to that of the other vaccines. We performed an analysis of synonymous versus nonsynonymous substitution rates [121], but the results were meaningless for many ORFs that had either no synonymous or no nonsynonymous substitutions (eg, in the C and H ORFs) (Figure 4).

### Variability in Individual Proteins

Table 1 lists the nucleotide and amino acid substitutions that are specifically discussed in the text. For a complete list of substitutions in the genomes of measles vaccines, see Supplemental Table 1. With the exception of Shanghai-191, the N genes of the vaccine strains show much higher conservation than the P/V/C, M, and H genes. Comparable to the situation in wild-type isolates [122], $N_{CORE}$ is much more conserved among the vaccines (only 4 cumulative nucleotide changes in the region coding for the amino terminal 400 amino acids for all the vaccines) than $N_{TAIL}$ (12 nucleotide changes in the region coding for the carboxyl terminal 125 amino acids) (Supplemental Table 1). Although the number of substitutions in Shanghai-191 is generally similar to that of other strains (except CAM-70), the N gene of Shanghai-191 is considerably more divergent than the N genes of all other viruses (Figure 4). The reason for the variability of the Shanghai-191N gene is that it accumulated 8 T→C changes, all of which are unique to Shanghai-191 (Supplemental Table 1). All of the T→C changes are located in the hypervariable $N_{TAIL}$ region, and 2 of them predict substitution with Pro residues that may affect protein conformation. The T→C changes may be an example of hypermutation in a vaccine strain. Hypermutation has been described for sequences of M genes derived from patients with SSPE [123]. The effect has been attributed to the action of the

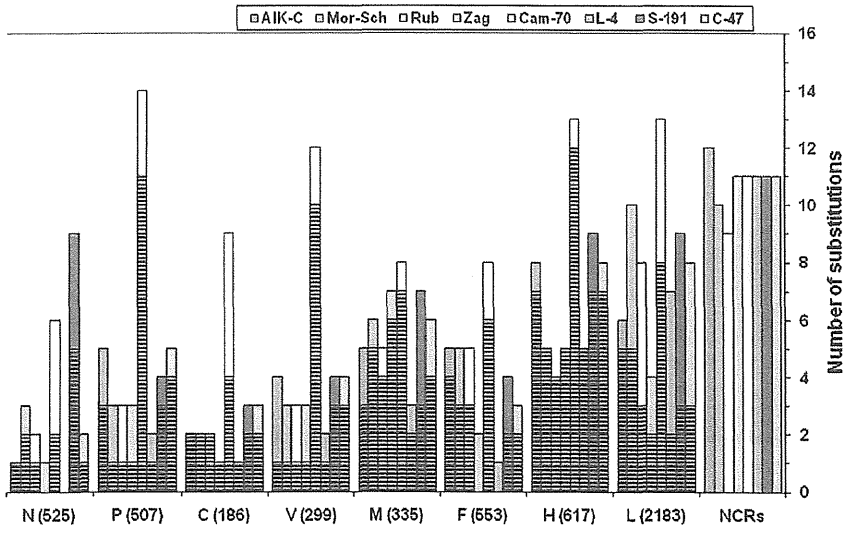


**Figure 4.** Number of nucleotide and predicted amino acid changes in the coding and noncoding regions of measles vaccine strains, compared with Edmonston wild-type strain. The number of amino acids is listed in brackets behind each protein. Horizontally striped bars represent coding changes, and open bars represent noncoding or silent changes. NCR, noncoding region.

**Table 1. Nucleotide and Amino Acid Substitutions Discussed in the Text**

| Gene | Nucleotide | Edmonston wild-type | Vaccine | Amino acid position | Edmonston wild-type | AIK-C | Mor-Sch | Rub | Zag | CAM-70 | L-4 | S-191 | C-47 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **26** | A | T | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | **42** | A | C | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| N | 722 | A | G | — | — | | | ● | | | | | |
| P | 1806 | G | A | — | — | | ● | ● | | ● | | | |
| | **2046** | C | T | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | 2063 | G | T | 86 | S | | | | | | | I | |
| | 2101 | A | G | 99 | N | | | | | D | | | |
| | 2134 | T | C | 110 | Y | | | | | | | | H |
| | 2135 | A | G | 110 | Y | | | | | C | | | |
| | 2167 | A | G | 121 | K | | | | | E | | | |
| | **2480** | A | G | 225 | E | G | G | G | G | G | G | G | G |
| | 3122 | T | C | 439 | L | P | | | | | | | |
| V | **2046** | C | T | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | 2063 | G | T | 86 | S | | | | | | | I | |
| | 2101 | A | G | 99 | N | | | | | D | | | |
| | 2134 | T | C | 110 | Y | | | | | | | | H |
| | 2135 | A | G | 110 | Y | | | | | C | | | |
| | 2167 | A | G | 121 | K | | | | | E | | | |
| | **2480** | A | G | 225 | E | G | G | G | G | G | G | G | G |
| C | **2046** | C | T | 73 | A | V | V | V | V | V | V | V | V |
| M | **3619** | G | A | 61 | G | D | D | D | D | D | D | D | D |
| | 3627 | C | T | 64 | P | | S | S | | S | | | |
| | **3702** | G | A | 89 | E | K | K | K | K | K | K | K | K |
| | **4232** | G | A | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | 4608 | T | C | — | — | | | ● | | | | | ● |
| F | **4978** | T | C | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | **5073**[a] | C | G | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | 5308 | T | C | — | — | | | ● | | | | | |
| | **5349** | T | C | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | **5514** | G | A | — | — | ● | ● | ● | ● | ● | ● | ● | ● |
| | 6542 | C | A/T[b] | 362 | S | Y (A) | Y (A) | Y (A) | | F (T) | | | |
| | 6743 | C | T | 429 | T | | | I | | | | | |
| | 7243 | T | C | — | — | | ● | | | | | | |
| H | **7407** | C | T | 46 | S | F | F | F | F | F | F | F | F |
| | **7901** | A | G | 211 | S | G | G | G | G | G | G | G | G |
| | **8711** | A | T | 481 | N | Y | Y | Y | Y | Y | Y | Y | Y |
| | 8721 | C | A | 484 | T | N | | | N | | N | N | N |
| | **8906** | G | A | 546 | G | S | S | S | | S | S | S | S |
| L | 9936 | A | G | 235 | I | | | | | V | | | |
| | 12,600 | A | C | — | — | | | ● | | | | | |
| | **14,383** | A | C | 1717 | D | A | A | A | A | A | A | A | A |
| | **14,579** | A | G | — | — | ● | ● | ● | ● | ● | ● | ● | ● |

**NOTE.** For abbreviations of virus names, see Figure 3. Dashes denote noncoding region, empty fields denote no change, compared with Edmonston wild-type, and dots denote substitution in noncoding region. Nucleotides highlighted in bold indicate a substitution in at least 8 vaccine strains.

[a] Correction to GenBank entry: Nucleotide 5073 in the GenBank entry for Edmonston wild-type (AF266288) is a G; the nucleotide was determined to be a C, as reported elsewhere [95] (B. Bankamp, unpublished data).

[b] Nucleotide positions with a mix of substitutions list the nucleotide in brackets behind the amino acid. For a list of all nucleotide and amino acid substitutions, please see Supplemental Table 1.

cellular double-stranded RNA-dependent adenosine deaminase [124], and the resulting defects in M protein function or expression contribute to the cell-associated growth pattern of SSPE viruses [125]. As the SSPE cases demonstrate, viruses with defective M proteins can replicate; however, the N protein is indispensable for replication. Although the N protein of Shanghai-191 is obviously functional, it is not known whether the large number of substitutions contributes to the strain's attenuation.

The Leucine residues that are important for the interaction of N with the M protein [21] are conserved in all strains. The $N_{TAIL}$ regions of vaccine strains and Edmonston wild-type possess Asn522, whereas currently circulating wild-type strains mostly possess Asp522 [29]. Asn522 is important for interaction with heat shock protein 72 (hsp72) [29]. This difference appears to reflect differences between genotypes rather than between vaccine and wild-type strains, because other genotype A, wild-type isolates share an Asn at position 522 (GenBank accession numbers AF045218 and AY647965). Although a recombinant MeV possessing Asp522 demonstrated reduced fitness in cell culture and cotton rats [29], the fact that current patient isolates share the Asp at this position indicates that these model systems may not reproduce all fitness pressures that wild-type viruses experience in the natural host. In addition to hsp72 and the M protein, $N_{TAIL}$ interacts with other binding partners, including several cellular proteins; however, no specific amino acids that are important for the interactions have been identified.

Nucleotide substitutions are often observed in the P gene after passages of MeV in cultured cells, and the P genes of wild-type viruses demonstrate a higher level of variability than the corresponding N genes [126–131]. Remarkably, 35 of 42 cumulative changes in the P gene are located in the amino-terminal domain shared between the P and V proteins (Supplemental Table 1). The carboxyl termini of both P and V are well conserved. These observations agree with data from wild-type MeVs in that the shared amino terminal domain of V and P was found to be more variable than the carboxyl termini in wild-type MeV isolates [132]. The unique carboxyl termini of P and V are required for replication and for control of the innate immune response, respectively; the higher level of conservation indicates that these regions are less tolerant of amino acid substitutions. Two amino acid substitutions, Tyr110His and Cys272Arg, which are found in the common amino-terminal region of the P and V proteins and the unique carboxyl-terminal region of the V protein, respectively, disrupt the abilities of the V and P proteins to counteract the IFN signaling pathways [34, 36, 43, 133]. Adaptation of a wild-type virus to chick embryo fibroblasts introduced the Tyr110His substitution, indicating that this substitution may be important for improved growth in avian cells [127]. The P/V proteins of the Changchun-47 vaccine possess Tyr110His, and the P/V proteins of the CAM-70 vaccine possess Tyr110Cys. The latter substitution reduced the ability of the CAM-70 V protein to counteract IFN signaling [43]. That the same position was changed in 2 independently derived vaccine strains through different nucleotide substitutions may indicate the importance of impeding IFN resistance in vaccine attenuation. The P protein (and presumably also the V protein) is phosphorylated at several residues in its amino-terminal domain, including amino acids 86, 151, and 180 [134]. The P and V proteins of Shanghai-191 possess a Ser86Ile substitution, which would abolish phosphorylation of this residue. The effect that this potential change in phosphorylation may have on the functions of P and V is currently unknown. One substitution that affects all vaccine strains is located at nucleotide 2480 in the P gene. This not only introduces a nonconservative Glu225Gly substitution at the end of the shared P/V domain—it also is located only 11 nucleotides upstream of the editing site. However, there was no significant difference in the frequency of P and V ORFs in transcripts from viruses with either nucleotide at position 2480 [132].

The P protein of CAM-70 varies from Edmonston wild-type at 11 amino acid positions and the V protein in 10 positions, while other vaccines vary at no more than 4 positions for the P protein and 3 for the V protein (Figure 4 and Supplemental Table 1). Many of these substitutions are nonconservative changes, including charge-switches at amino acids 99 and 121. Although the effect of the substitution at amino acid 110 in the P/V protein of CAM-70 on the inhibition of IFN signaling has been demonstrated [43], the contribution of the many other substitutions to attenuation remains to be investigated. The P protein of AIK-C renders the virus temperature-sensitive [135]; a Leu439Pro substitution was found to be one contributing factor [136]. None of the other vaccines share this substitution.

There are a cumulative total of 25 nucleotide substitutions and 18 amino acid changes in the small C ORF (186 amino acids), with a high proportion of coding changes (72%) (Supplemental Table 1). Such a high level of variability may indicate the importance of the C protein as a virulence factor. The C ORF of CAM-70 has 9 nucleotide changes, compared with only 0-3 in the other vaccines; however, the number of silent changes is high (Figure 4). As a result, the number of amino acid substitutions in CAM-70 C is 4, whereas the other vaccines have 0-2 substitutions. The high number of silent changes in the CAM-70 C ORF is a result of coding changes in P and V proteins. The C protein interferes with the induction of IFN and apoptosis through down-regulation of transcription [35, 42]. Using a mini-replicon assay system, Bankamp et al [45] reported that the inhibitory activity of the C protein toward viral RNA synthesis differed among 6 MeV strains and that substitutions in the region between amino acids 46 and 167 of the C protein affected its ability to down regulate viral transcription. All but 1 of the substitutions in the C proteins of the vaccine strains are located in this region (Supplemental Table 1), so it is possible that some of them may affect the ability of the C protein to control the induction of IFN. After adaptation of a wild-type virus to CEF, the C protein contained 1 amino acid change that affected its ability to down-regulate transcription [126], providing additional evidence of the importance of the C protein in cell-culture adaptation. Disturbing the delicate balance between the rate of replication and inhibition of host defenses may lead to attenuation. Because the C proteins are important virulence factors [90, 137–140], the differences among the C proteins may also contribute to the attenuated phenotypes of certain measles