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# Characterization of measles virus strains circulating in Southern Italy (Palermo area, Sicily) between 2010 and 2011

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ABSTRACT

Measles virus (MV) was classified in 24 genotypes that show a distinct geographic distribution. Genotypes contain multiple distinct lineages. In 2011 large outbreaks of measles occurred in Italy and in many European countries. Aims of this study are to analyze the intra-genotype variability and to follow the importation and the spread of new MV strains in Sicily. A fragment of 450 bps of MV C-terminal nucleoprotein was sequenced from sera of 73 Sicilian patients with symptomatic measles infections, occurred between 2010 and 2011. Five MV strains were D4 genotype and 68 were D8 genotype. The MV/D4 sequences were related to MV/D4-Enfield variant. Two lineages of MV/D8 genotypes, related to MV/D8-Villupuram variant and to a strain found in Birmingham in 2006 respectively, were identified. This is the first study that reports the co-circulation of different MV genotypes and lineages in Sicily suggesting multiple origins of the outbreak that occurred during 2010 and 2011 years.


## 1. Introduction

Measles virus (MV) infection is responsible for erythematous and maculopapular rash disease. In up to 40% of patients with measles disease some complications as pneumonia, laryngotracheobronchitis and otitis media may occur. Rare complications are the post-measles encephalitis to autoimmune etiology. Furthermore in immunosuppressed children, months after measles rash, was described a measles inclusion body encephalitis consequential to persistent infection.

Thanks to the availability of a safe and effective measles vaccine, morbidity and mortality rates have been dramatically reduced since 1963 (Murray et al., 2003). The World Health Organization (WHO) had set year 2010 as the target for elimination of measles in Europe, but then this goal has been postponed to 2015. Measles elimination was defined as the interruption of indigenous MV transmission for a twelve-month period. The necessary conditions for the elimination of the virus are the measles disease incidence less than 1 per 1,000,000 inhabitants, 0 cases of measles caused by endemic virus for a least 12 consecutive months and vaccination rates >95% (WHO Guidelines, 2012). Measles virus is a monotypic virus of Morbillivirus genus of Paramyxoviridae family. Based on the variability of 450 bps of C-terminal nucleoprotein (N-450) MV was classified in 24 genotypes that show distinct geographic distributions. Globally, the MV genotype B3 (MV/B3) is prevalent in Africa Region, MV/B3-D4-D5-D8 co-circulating in the Americas, MV/B3-D4-D8 in the Eastern Mediterranean Region, MV/B3-C2-D4-9-H1 in European region and the genotypes MV/D4-D5-D8-D9-D11-G2-G3-H1 in Asia region (Rota et al., 2011). Genotypes contain multiple distinct lineages. A lineage is defined as a group of homologous viruses for N-450 sequence and this group is probably linked to a single chain of transmission. In measles endemic countries, one or few genotypes are in continuous circulation, while multiple genotypes and lineages are usually detected where the incidence is very low and the measles cases are due to importation (Rota et al., 2009).

Molecular genotyping of MV, combined with epidemiological data, allows to prove the importations of new strains, to identify the transmission pathways and also to illustrate the progress made towards elimination. Although in Italy, since 1999, the measles vaccine in combination with rubella and mumps, was included in the national immunization program, and since 2003 a novel two dose schedule (at 12–15 months and at five years) was approved in all Italian regions, the vaccination coverage is still below 95%, which is the target of the National Measles Elimination Plain. Because of the incomplete vaccination coverage and the presence of a wide susceptible population of adolescents and young adults, many cases of measles infection are taking place in Italy.

Before 2007, the genetic analysis of measles isolates showed the co-circulation of numerous genotypes including genotype A, closely related to measles vaccine strains. This condition is typical in countries where the new cases of MV infection are due to imported strains. After 2007 the MV/D4 genotype was isolated from more than the 78% of measles cases, while genotype D8 from 19% of cases. Data on MV isolates circulating in Sicily, are restricted to few information related to the

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2002–2007 period, in which three MV isolates, one of B3 genotype and 2 of D4, were identified (Magurano et al., 2012).

In 2011 a large outbreaks of measles occurred in many European countries. In Italy, an incidence of 7.7 cases per 100,000 population was registered and then it decreased at 1.0 and 3.8 per 100,000 in 2012 and 2013 respectively (Magurano et al., 2015).

Aim of this study is to identify MV genotypes responsible of 117 cases of measles disease from a wide outbreak occurred between 2010 and 2011. The study of N-450 sequences was used to analyze the intra-genotype variability and to follow the importation and the spread of new strains in Sicily, a region of southern Italy in the middle of Mediterranean basin.

## 2. Materials and Methods

### 2.1. Samples

During a measles outbreak, serum samples of 117 patients, enrolled consecutively with symptomatic measles disease and serologically confirmed (IgM antibodies positive) referred to the Infectious Disease of the University of Palermo between March 2010 and October 2011, were included in this study. Of the patients studied, 75 were men, 42 were women; mean age 25, range 13–44 years. Moreover, ten serum samples of sporadic measles disease collected from 2009 were included. All patients were Sicilian and none had been vaccinated against measles. The study protocol was approved by the Ethics Committee of the hospital and written consent for collection of clinical data and sequence analysis of MV was obtained from all patients.

### 2.2. Amplification

Viral RNA was extracted from 140 μl of serum using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. A fragment of 580 bp encoding the 3′ terminal of Nucleoprotein gene was obtained by an in-house RT nested PCR with minor changes (Alla et al., 2006). The first round of PCR was done with the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), according to manufacturer's instructions. RT-PCR cycling conditions consisted of an initial incubation at 50 °C for 62 min, a hot-start at 94 °C for 2 min, 35 cycles, each at 94 °C for 30 s, at 55 °C for 45 s, and at 72 °C for 45 s, and a final step at 72 °C for 7 min. 2 μl of first-round PCR product were added to 48 μl of reaction mixtures containing the Accu Prime Pfx DNA polymerase system (Invitrogen, Carlsbad, USA). The nested PCR thermal cycle was characterized by a hot-start at 94 °C for 5 min, followed by 35 cycles, each at 95 °C for 30 s, at 47 °C for 30 s, and at 72 °C for 60 s, and by a final extension at 72 °C for 7 min. Standard procedures for reducing contamination were strictly followed.

### 2.3. Analysis of measles sequence

The nucleotide sequence was determined by direct sequencing. Approximately 15 ng of purified DNA were labelled by Big Dye Terminator v 1.1 and analysed by a sequencer ABI Prism 3100 instrument (Applied Biosystems, Foster City, USA). All sequences were aligned based on viral genotype with the Clustal W algorithm integrated into the BioEdit software, and the phylogenetic tree was constructed with the Mega 6 program using the Kimura two-parameter system and the Neighbor-joining method (bootstrap 1000 replicates). Seventy-eight World representative sequences of MV genotype D4 and D8 obtained from GenBank (GenBank accession Nos.: AB678711; AB786669; AB983348; AF280801; AF280803; AF410991; AF481490; AJ250071-72; AM778841; AM849093; AY037034; AY249250; AY521172; DQ023699; DQ390238; DQ390245; DQ390257; DQ398060; DQ398066; DQ400389; DQ779206; DQ852616; DQ917466; DQ917468; DQ917480; EF017348; EF079124; EF079136; EF121965; EF179548; EF428178; EF533887; EF554312; EF6000554; EF607927; EU086729; EU139103; EU139092; EU439426; EU812284; EU925764; FJ595982; FJ475060; FJ790767; FJ831441; FJ361901; FJ65078; HM046483; HM067829; HM173091; HM196167; HM214468; HM240848; HM440232; HM581906; HM802120; HQ436108; HQ453176; JF681233; JF911794; JF937920; JQ246498; JQ341200; JQ417687; JQ783007; JQ783053; JX402873; JX559403; KC117298; KF47709; KJ411829; KJ437156; KJ586230; KJ659722; KJ659756; KJ659739; KM878568.) were used to confirm the MV genotype and to conduct phylogenetic analysis.

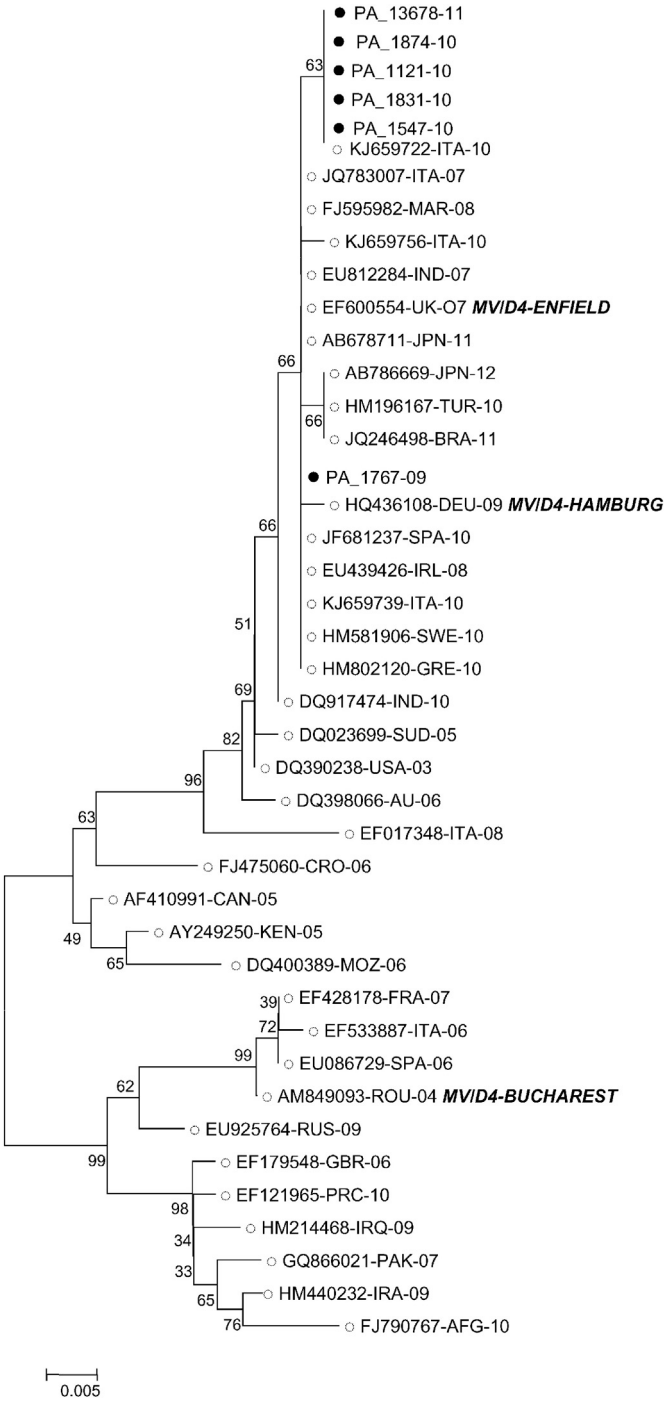


**Fig. 1.** Phylogenetic tree of N-450 sequences of MV/D4 genotype. Neighbor-joining tree of N-450 sequences constructed using Kimura 2-parameter with boostrap values of 1000 replicates. The tree contained 33 sequences of measles D4 genotype, referred in GenBank, indicated by empty circles (○) followed by Accession Nos-Country-Year, and 6 MV-D4 isolates from Palermo patients, indicated by filled circles (●) followed by PA_n° of isolate-year. Well-known variants were written in bold.