Rev Iberoam Micol. 2014;31(1):49–53

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Mycologic Forum

Molecular markers in the epidemiology and diagnosis of coccidioidomycosis

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ARTICLE INFO

Article history:
Received 27 August 2013
Accepted 27 September 2013
Available online 20 November 2013

Keywords:
Coccidioides spp.
Genetic variability
Molecular markers

ABSTRACT

The prevalence of coccidioidomycosis in endemic areas has been observed to increase daily. To understand the causes of the spread of the disease and design strategies for fungal detection in clinical and environmental samples, scientists have resorted to molecular tools that allow fungal detection in a natural environment, reliable identification in clinical cases and the study of biological characteristics, such as reproductive and genetic structure, demographic history and diversification. We conducted a review of the most important molecular markers in the epidemiology of *Coccidioides* spp. and the diagnosis of coccidioidomycosis. A literature search was performed for scientific publications concerning the application of molecular tools for the epidemiology and diagnosis of coccidioidomycosis. The use of molecular markers in the epidemiological study and diagnosis of coccidioidomycosis has allowed for the typing of *Coccidioides* spp. isolates, improved understanding of their mode of reproduction, genetic variation and speciation and resulted in the development specific, rapid and sensitive strategies for detecting the fungus in environmental and clinical samples. Molecular markers have revealed genetic variability in *Coccidioides* spp. This finding influences changes in the epidemiology of coccidioidomycosis, such as the emergence of more virulent or antifungal resistant genotypes. Furthermore, the molecular markers currently used to identify *Coccidioides immitis* and *Coccidioides posadasii* are specific and sensitive. However, they must be validated to determine their application in diagnosis.

This manuscript is part of the series of works presented at the "V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi" (Oaxaca, Mexico, 2012).

Marcadores moleculares en la epidemiología y el diagnóstico de la coccidioiodomicosis

Palabras clave:
Coccidioides spp.
Variabilidad genética
Marcadores moleculares

RESUMEN

Se ha descrito un aumento constante de la prevalencia de coccidioidomicosis en zonas endémicas. Para conocer las causas de esta expansión de la enfermedad y planificar estrategias para la detección del hongo en muestras clínicas y ambientales, se ha recurrido al uso de instrumentos moleculares que permitan la detección de estos hongos en su ambiente natural, su identificación fiable en los casos clínicos y el estudio de sus características biológicas, historia demográfica, diversificación y estructura reproductora y genética. El presente estudio representa una revisión de las implicaciones más importantes de los marcadores moleculares en la epidemiología de *Coccidioides* spp. y el diagnóstico de la coccidioidomicosis. Para ello, se efectuó una búsqueda de los artículos publicados sobre la aplicación de los instrumentos moleculares en la epidemiología y el diagnóstico de la coccidioidomicosis. El uso de marcadores moleculares en el estudio de la epidemiología y el diagnóstico de la coccidioidomicosis ha permitido tipificar aislamientos de *Coccidioides* spp., conocer su modo de reproducción, variabilidad genética y su especiación, así como la planificación de estrategias más rápidas, específicas y sensibles para detectar el hongo en muestras ambientales y clínicas. Los marcadores moleculares han revelado la variabilidad genética de *Coccidioides*, hallazgo importante porque puede influir en la epidemiología de la coccidioidomicosis,

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1130-1406/$ – see front matter
http://dx.doi.org/10.1016/j.riam.2013.09.011

como la aparición de genotipos más virulentos o resistentes a los antimicóticos. Por otro lado, los marcadores moleculares para la identificación de *Coccidioides immitis* y *Coccidioides posadasii*, descritos hasta la fecha, son específicos y sensibles; sin embargo, deben validarse para determinar su aplicación en el diagnóstico.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

The genus *Coccidioides* consists of two closely related species, *Coccidioides immitis* and *Coccidioides posadasii*. Both species have been classified in the Onygenales order and Ascomycota phylum. *Coccidioides* is a dimorphic pathogen that grows as a filamentous saprobe in soil when not infecting mammals. This phase is very resistant, which allows the fungus to remain viable in arid or semi-arid soils for months or years. Humid soil favors fungal growth, which gives rise to the formation of asexual propagules (arthroconidia). The wind disperses the arthroconidia and they can later be inhaled by humans or animals.[15,28] Once in the host, the arthroconidia become spherules, which is a morphological change of polarized isotropic growth. Spherules subsequently differentiate to produce internal spores (endospores). When the spherules rupture, the endospores are released and are able to propagate in the host and restart the spherulation cycle, a process that may result in systemic fungal infection in humans and other vertebrates.[4] Previously, *C. immitis* was the only known causal agent of coccidioidomycosis. However, after 2002, based on microsatellite analysis, it was concluded that this species includes two different taxa: *C. immitis*, which is endemic in California and *C. posadasii*, which is endemic in the southwestern part of the United States of America and northern Mexico, as well as Central and South America.[16] Currently, an increasing number of outbreaks have occurred in endemic areas, which has generated interest in studying these fungi and their genetic and reproductive characteristics, demographic history and diversification process. Depending on the purpose of study, several molecular markers, alone or in combination, have been used in the development of specific and sensitive strategies for the diagnosis of coccidioidomycosis.

Molecular markers

Molecular markers are a dataset collected from various molecular techniques and represent unique genetic traits in individuals, populations or species. Variants of these genetic traits are the result of randomly occurring mutations or mutations influenced by the environment. The latter results in a genetic variation (polymorphism) and is essential for the adaptation to environmental changes and, therefore, the survival of the species.[24]

In order to detect genetic differences in the molecular markers, several properties must be present: whether variable or polymorphic, the genetic change must have a defined inheritance pattern, be observed frequently in the genome, occur under neutral selection (not influenced by the environment), be easily accessed, and have high reproducibility. Because it is extremely difficult to find a molecular marker that meets all the above criteria, some authors use combinations of different molecular markers, as they are most informative.[24]

Molecular markers have been used in the study of coccidioidomycosis to identify species, type isolates, determine the structure or reproductive mode and identify the degree of genetic differentiation between isolates and species. At the clinical level, knowledge of the genetic variability of the *Coccidioides* species, allows for the development of antifungal agents and vaccines, because their design will take into account genetic diversity, thus ensuring that the action of these biological products reliable covers all genotypes of the species. Furthermore, the use of molecular markers is an alternative to improve the diagnosis of coccidioidomycosis, because conventional methods for detecting and identifying the fungus are time-consuming and less sensitive and specific.

Role of molecular markers in the epidemiology of coccidioidomycosis

Genetic variability of Coccidioides spp.

The first studies identifying genotypic variability in populations of the *Coccidioides* genus only included *C. immitis*; *C. posadasii* was not formally recognized until 2002. The first genotypic method used to analyze the genetic variability and related isolates of *C. immitis* was described by Zimmermann et al.[38] In this study, they used Restriction Fragment Length Polymorphic (RFLP) from DNA obtained from 15 patient-isolates in California. Their results showed a similar RFLP pattern in 13 of the 15 isolates and a second pattern in the remaining two isolates, which indicated genetic diversity between the two isolates and the remainder of the isolates studied. RFLP involves obtaining DNA fragments by restriction endonuclease digestion. These fragments may vary in size and number, enabling the observation of polymorphisms among the isolates.

Furthermore, Burt et al.[9] studied the pattern of genetic variation in 25 isolates of *C. immitis* from a hospital in Tucson, Arizona, using PCR amplification and Single-Strand Conformational Polymorphism (SSCP) detection. Their results also showed genetic variation among the examined isolates. In SSCP, double-stranded fragments are denatured by heating and then cooled to prevent further association. Single chain molecules of DNA can form secondary structures due to internal base pairing. These differences in secondary structure cause the DNA strands to migrate differently during electrophoresis with non-denaturing acrylamide. The variation in electrophoretic mobility of single-stranded DNA is likely due to changes caused by nucleotide substitutions. The variable bands are extracted from the gel and sequenced. When polymorphic regions are found, new oligonucleotides are designed and used to amplify the fragments from each isolate studied.[13]

In addition, Burt et al.[11] utilized DNA Multilocus Sequence Typing (MLST) of *C. immitis* isolates from Arizona, California and Texas, to obtain information about the structure of fungal populations. There was evidence of genetic differentiation among the three populations studied, which suggests a very low level of gene flow between them. MLST involves the PCR amplification of fragments (450–500 bp) of various housekeeping genes (7–8 genes), followed by sequencing both strands (alleles) to observe sequence changes. Analysis of changes in the fungal housekeeping genes allows for typing populations or isolates based on their allelic profiles.[1]

Recently, Sharpton et al.[32] and Neafsey et al.[25] used Whole Genome Shotgun (WGS) sequencing of *C. immitis* and *C. posadasii*