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Multilocus sequence typing analysis reveals that *Cryptococcus neoformans* var. *neoformans* is a recombinant population

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ABSTRACT

Cryptococcus neoformans var. *neoformans* (serotype D) represents about 30% of the clinical isolates in Europe and is present less frequently in the other continents. It is the prevalent etiological agent in primary cutaneous cryptococcosis as well as in cryptococcal skin lesions of disseminated cryptococcosis. Very little is known about the genotypic diversity of this *Cryptococcus* subtype. The aim of this study was to investigate the genotypic diversity among a set of clinical and environmental *C. neoformans* var. *neoformans* isolates and to evaluate the relationship between genotypes, geographical origin and clinical manifestations. A total of 83 globally collected *C. neoformans* var. *neoformans* isolates from Italy, Germany, France, Belgium, Denmark, Greece, Turkey, Thailand, Japan, Colombia, and the USA, recovered from different sources (primary and secondary cutaneous cryptococcosis, disseminated cryptococcosis, the environment, and animals), were included in the study. All isolates were confirmed to belong to genotype VNIV by molecular typing and they were further investigated by MLST analysis. Maximum likelihood phylogenetic as well as network analysis strongly suggested the existence of a recombinant rather than a clonal population structure. Geographical origin and source of isolation were not correlated with a specific MLST genotype. The comparison with a set of outgroup *C. neoformans* var. *grubii* isolates provided clear evidence that the two varieties have different population structures.

1. Introduction

Cryptococcus neoformans and *C. gattii* are two sibling yeast species responsible for cryptococcosis. This life-threatening disease is mainly associated with AIDS patients in the countries where the HIV infection burden is still high such as in sub-Saharan Africa and in South East Asia (Assogba et al., 2015; Park et al., 2009). In developed countries, however, the incidence of cryptococcosis in HIV-infected population is decreasing due to the introduction of high active antiretroviral therapy (HAART). In contrast, the disease is increasingly found in non-AIDS patients such as those with hematological neoplasms, recipients of organ transplantation, and victims of autoimmune diseases (Bratton et al., 2012; Henao-Martínez and Beckham, 2015; Sanchini et al., 2014).

C. neoformans is classified into two varieties, three serotypes and five molecular types. *C. neoformans* var. *grubii*, serotype A, is identified as the molecular types VNI, VNII and VNB, whereas *C. neoformans* var. *neoformans*, serotype D, belongs to molecular type

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Table 1
Clinical and molecular information of the 83 *C. neoformans* var. *neoformans* isolates investigated in the present study.

Strain code	Category	Origin	Source	Date	Underlying disease	Molecular type	Mating type	Sequence type	*CAP59*	*GPD1*	*IGS1*	*LAC1*	*PLB1*	*SOD1*	*URA5*	Reference
WM629	DC	Australia	Blood	1987	AIDS	VNIV	αD	**117**	16	21	30	19	13	1	19	*Meyer*
IUM 01-4729	ENV	Belgium	Pigeon droppings	2001	–	VNIV	αD	**510**	16	21	24	18	13	20	32	*Cogliati*
IUM 01-4730	ENV	Belgium	Dust	2001	–	VNIV	αD	**514**	16	40	24	20	13	20	32	*Cogliati*
IUM 97-4899	PPCC	Belgium	Skin	1997	No risk factors	VNIV	αD	**507**	16	3	26	39	14	20	34	*Cogliati*
IUM 98-5036	PPCC	Belgium	Skin	1998	Diabete	VNIV	αD	**506**	16	3	24	20	13	20	32	*Cogliati*
H0058-I-1406	DC	Colombia	CSF	2002	AIDS	VNIV	αD	**335**	27	28	30	19	14	17	41	*Escandon*
H0058-I-2250	DC	Colombia	CSF	2004	AIDS	VNIV	αD	**336**	16	22	32	14	14	18	17	*Escandon*
H0058-I-2291	DC	Colombia	CSF	2004	AIDS	VNIV	αD	**160**	16	21	30	19	13	17	19	*Escandon*
H0058-I-2880	DC	Colombia	CSF	2007	AIDS	VNIV	αD	**336**	16	22	32	14	14	18	17	*Escandon*
NIH-424	ENV	Denmark	Pigeon nest	1970	–	VNIV	αD	**180**	20	21	26	21	19	17	21	*Kwon-Chung*
NIH-429	ENV	Denmark	Pigeon nest	1970	–	VNIV	αD	**512**	16	21	31	19	13	19	16	*Kwon-Chung*
NIH-430	ENV	Denmark	Pigeon nest	1970	–	VNIV	**a**D	**509**	16	20	24	16	14	20	16	*Kwon-Chung*
NIH-433	ENV	Denmark	Pigeon nest	1970	–	VNIV	**a**D	**515**	17	21	28	13	14	17	16	*Kwon-Chung*
CNRMA00.330	PCC	France	Skin	2000	No risk factor	VNIV	αD	**135**	27	22	43	24	13	17	20	*Dromer*
CNRMA00.840	SCC	France	Skin	2000	Hematological malignancy	VNIV	αD	**125**	16	21	45	21	13	21	22	*Dromer*
CNRMA07.1501	SCC	France	CSF	2007	AIDS	VNIV	αD	**180**	20	21	26	21	19	17	21	*Dromer*
CNRMA97.697	DC	France	CSF	1997	AIDS	VNIV	αD	**121**	16	21	32	19	13	17	20	*Dromer*
CNRMA98.480	DC	France	CSF	1998	AIDS	VNIV	αD	**511**	16	21	29	16	14	17	24	*Dromer*
CNRMA99.1037	PCC	France	Skin	1999	No risk factor	VNIV	αD	**122**	16	21	32	24	13	17	20	*Dromer*
MKT6301	PCC	France	Skin	2011	No risk factor	VNIV	αD	**121**	16	21	32	19	13	17	20	*Bienvenu*
RKI 04-0061	DC	Germany	–	2004	Liver disorder	VNIV	αD	**110**	15	21	24	21	13	20	22	*Rickerts*
RKI 04-0089	PCC	Germany	Skin, hand	2004	Chronical asthma, corticosteroids	VNIV	αD	**486**	16	22	32	19	13	17	22	*Rickerts*
RKI 05-0151	DC	Germany	Blood	2005	Diabetes	VNIV	αD	**531**	16	29	24	20	13	20	18	*Rickerts*
RKI 07-0173	DC	Germany	–	2007	Liver disorder	VNIV	αD	**530**	31	21	83	13	37	59	53	*Rickerts*
RKI 08-0429	DC	Germany	CSF	2008	AIDS	VNIV	αD	**487**	16	21	32	24	13	17	32	*Rickerts*
RKI 08-0572	DC	Germany	–	2008	No risk factors	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 08-0591	DC	Germany	–	2008	No risk factors	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 09-0102	DC	Germany	–	2009	Sarcoidosis	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 09-0103	DC	Germany	–	2009	Sarcoidosis	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 09-0388	PCC	Germany	Skin	2009	No risk factor	VNIV	αD	**519**	26	22	32	24	13	17	20	*Rickerts*
RKI 09-0393	PCC	Germany	Skin	2009	No risk factor	VNIV	αD	**505**	15	21	31	15	13	19	18	*Rickerts*
RKI 09-0515	DC	Germany	BAL	2009	Solid organ Tx	VNIV	αD	**168**	22	21	30	22	14	17	18	*Rickerts*
RKI 09-0545	DC	Germany	CSF	2009	AIDS	VNIV	αD	**160**	16	21	30	19	13	17	19	*Rickerts*
RKI 11-0047	DC	Germany	CSF	2010	AIDS	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 11-0048	DC	Germany	CSF	2010	AIDS	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 12-0155	PCC	Germany	Skin	2012	No risk factor	VNIV	αD	**116**	16	21	29	13	14	17	24	*Rickerts*
RKI 12-0559	PCC	Germany	Skin	2012	No risk factor	VNIV	αD	**513**	16	21	43	21	13	20	18	*Rickerts*
RKI 13-0490	ENV	Germany	Pigeon droppings	2013	–	VNIV	**a**D	**522**	27	21	53	19	14	19	16	*Rickerts*
RKI 13-0491	ENV	Germany	Pigeon droppings	2013	–	VNIV	**a**D	**523**	29	21	83	21	14	58	53	*Rickerts*
RKI 13-0492	ENV	Germany	Pigeon droppings	2013	–	VNIV	**a**D	**524**	29	21	83	40	14	58	53	*Rickerts*
GRACA18BK1-3	ENV	Greece	Eucalyptus tree	2013	–	VNIV	**a**D	**502**	26	38	30	22	13	19	18	*Velegraki*
GRACP14BK1-1	ENV	Greece	Pine tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*
GRACP15SO1-1	ENV	Greece	Pine tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*
GRACP15SO1-2	ENV	Greece	Pine tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*
GRACP16HO1-1	ENV	Greece	Pine tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*
GRACP30BK1-1	ENV	Greece	Plane tree	2013	–	VNIV	**a**D	**498**	26	20	30	17	13	19	18	*Velegraki*
GRAKI10SO1-1	ENV	Greece	Olive tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*
GRAKI11HO1-1	ENV	Greece	Olive tree	2013	–	VNIV	**a**D	**499**	26	21	30	22	13	19	18	*Velegraki*

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