Oligonucleotide Array-CGH Identifies Genomic Subgroups and Prognostic Markers for Tumor Stage Mycosis Fungoides

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Mycosis fungoide (MF) patients who develop tumors or extracutaneous involvement usually have a poor prognosis with no curative therapy available so far. In the present European Organization for Research and Treatment of Cancer (EORTC) multicenter study, the genomic profile of 41 skin biopsies from tumor stage MF (MFt) was analyzed using a high-resolution oligo-array comparative genomic hybridization platform. Seventy-six percent of cases showed genomic aberrations. The most common imbalances were gains of 7q33.3q35 followed by 17q21.1, 8q24.21, 9q34qter, and 10p14 and losses of 9p21.3 followed by 9q31.2, 17p13.1, 13q14.11, 6q21.3, 10p11.22, 16q23.2, and 16q24.3. Three specific chromosomal regions, 9p21.3, 8q24.21, and 10q26qter, were defined as prognostic markers showing a significant correlation with overall survival (OS) ($P=0.042$, 0.017, and 0.022, respectively). Moreover, we have established two MFt genomic subgroups distinguishing a stable group (0–5 DNA aberrations) and an unstable group (>5 DNA aberrations), showing that the genomic unstable group had a shorter OS ($P=0.05$). We therefore conclude that specific chromosomal abnormalities, such as gains of 8q24.21 (*MYC*) and losses of 9p21.3 (*CDKN2A*, *CDKN2B*, and *MTAP*) and 10q26qter (*MGMT* and *EBF3*) may have an important role in prognosis. In addition, we describe the MFt genomic instability profile, which, to our knowledge, has not been reported earlier.

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Abbreviations: BAC, bacterial artificial chromosome; CGH, comparative genomic hybridization; DB, DNA breaks; FISH, fluorescence in situ *hybridization; HD, homozygous deletion; HLA, high level amplification; MFt, tumor stage mycosis fungoides; OS, overall survival*

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INTRODUCTION

Mycosis fungoides (MF) is a low-grade mature T-cell neoplasm of malignant CD4-positive helper T lymphocytes with a marked affinity for the skin, particularly the epidermis. It is the most frequent type of cutaneous T-cell lymphoma with an annual incidence close to 0.9 per 100,000 individuals in the United States (Criscione and Weinstock, 2007). MF has a long natural evolution over the years or sometimes decades and develops in a multistep process from patches to more infiltrated plaques and eventually tumors (tumor stage MFs, MFt). Although patients with patch or plaque disease normally have a long survival, those cases developing tumors or extracutaneous involvement usually have a poor prognosis with no curative therapy available so far.

To characterize the genetic profile and to identify prognostic markers for MFt, several comparative genomic hybridization (CGH) studies (Karenko *et al.*, 2003; Mao *et al.*, 2002; Mao *et al.*, 2003; Fischer *et al.*, 2004; Prochazkova *et al.*, 2007) have been reported. Although conventional CGH allows the identification of chromosomal imbalances, the identification of specific genes involved in the prognosis remains elusive because of the low resolution of this technique (5–10 Mb). In addition, most of the prognostic

markers in MF identified by different CGH studies have a limited discriminatory power because of the fact that the number of patients in this specific stage is too small. Furthermore, the different series studied are heterogeneous (Karenko *et al.*, 2002; Mao *et al.*, 2002; Mao *et al.*, 2003; Fischer *et al.*, 2004), including a mixture of patients belonging to different cutaneous lymphoma entities (MF, Sezary syndrome (SS), primary cutaneous anaplastic large cell lymphoma, and lymphomatoid papulosis), which actually have very different clinical outcomes making it very difficult to analyze the prognostic implication of the results. The development of genome-wide analysis techniques (Solinas-Toldo *et al.*, 1997; Pinkel *et al.*, 1998; Pinkel and Albertson, 2005) has allowed the characterization of more precisely several human neoplasms with the aim of providing prognostic markers and targets for directed therapeutic intervention. More recently, van Doorn *et al.* (2009) reported a bacterial artificial chromosome (BAC)arrayCGH study in which genomic differences between SS and MFt were identified. Although BACarrayCGH allows the identification of DNA copy number changes, it does not offer a straightforward and reliable detection of small alterations because of the larger-sized BAC probes. Thus, the identification of specific genes that are involved could remain a challenge (Gunnarsson *et al.*, 2008).

The aim of this study was to analyze genetic abnormalities in MFt patients using a 60-mer 44K oligonucleotide-arrayCGH platform to characterize this entity in a large series of patients. Furthermore, we evaluated whether specific genetic alterations may provide prognostic information.

RESULTS

Array CGH results and genomic instability profile

Among the 41 MF patients, 32 (78%) showed aberrant profiles by arrayCGH analysis, whereas no chromosomal abnormalities were detected in nine cases (22%). All the alterations detected are summarized in Supplementary Table S1. Losses were more frequently observed than gains (63.3 *vs* 36.7%) and the mean chromosomal imbalances per case were 3.3 gains (range 0–14) and 5.7 losses (range 0–30). The most frequent alterations are presented in Table 1. Regarding chromosomal aberrations, the highest frequency of gains was detected on 7q33.3q35 followed by 17q21.1, 8q24.21, 9q34qter, and 10p14. The most frequent deletions were observed on chromosome 9p21.3 followed by 9q31.2, 17p13.1, 13q14.11, 6q21.3, 10p11.22, 16q23.2, and 16q24.3 (Table 1). Global results are summarized in Figure 1a. Interestingly, five homozygous deletions (HDs) and five high-level amplifications (HLAs) have been detected. The sizes of the aberrations mentioned and of the candidate genes mapped in these regions are given in Supplementary Table S2. Certain chromosomal abnormalities detected by arrayCGH were confirmed by fluorescence *in situ* hybridization (FISH) (patients 4, 8, and 29; Figure 1b).

In addition, data obtained by (Conde *et al.*, 2007) oligonucleotide-arrayCGH and InSilico arrayCGH software provided a global genomic profile for MFt patients that was analyzed in terms of genomic instability. This analysis has allowed the segregation of MFt patients into two major subgroups. The first subgroup, called genomically stable MFt, included 18 cases. It was characterized by a low number of changes (0–5), low presence of DNA breaks (DBs) (0 (0;3)), and the absence of HLA and HD. The second group, called genomically unstable MFt, with 23 cases, displayed a higher number of genomic abnormalities. It was characterized by a high number of changes (6–34), DBs (14 (10;21)), and the presence of HLA and HD. The multiple testing between the genomic status and the most prominent smallest overlapping region of imbalances found in MFt patients has shown a significant relation between the genomic status and the gain

Table 1. Summary of the most frequent prominent alterations in MFt patients

Type of change	Start gene	Chr	Cytoband	Size (Mb)	% Patients	Candidate genes
Gains	BG495318	7	q33.3q35	14.2	55	*PTN, HIPK2, BRAF, TRPV6, TRPV5, PIP, EPHA1, EZH2*
Gains	SMARCE1	17	q21.1	4.7	37	*STAT5A, STAT5B, STAT3*
Gains	M13930	8	q24.21	0.75	32	*MYC*
Gains	SLC2A8	9	q34	11	17	*NOTCH, TRAF2, CARD9*
Gains	chr10: 004083817	10	p14	4.73	17	*GATA3, IL2R*
Gains	chr1:195487682	1	q31.2q32.2	7.7	15	*KIF14*
Losses	MTAP	9	p21.3	0.2	42	*MTAP, CDKN2A, CDKN2B*
Losses	SLC35D2	9	q31.2	3.9	30	*CDC14B, XPA, NR4A3*
Losses	DULLARD	17	p13.1	1.02	27.5	*TP53, TNK1*
Losses	chr13:047357604	13	q14.11	2.22	20	*RB*
Losses	CDC2L6	6	q21.3	1.2	17	No genes related to cancer
Losses	chr10:031132968	10	p11.22	1.5	17	*TCF8*
Losses	BQ189302	16	q23.2	6.7	17	*CDH13*
Losses	ZNF469	16	q24.3	0.78	17	No genes related to cancer

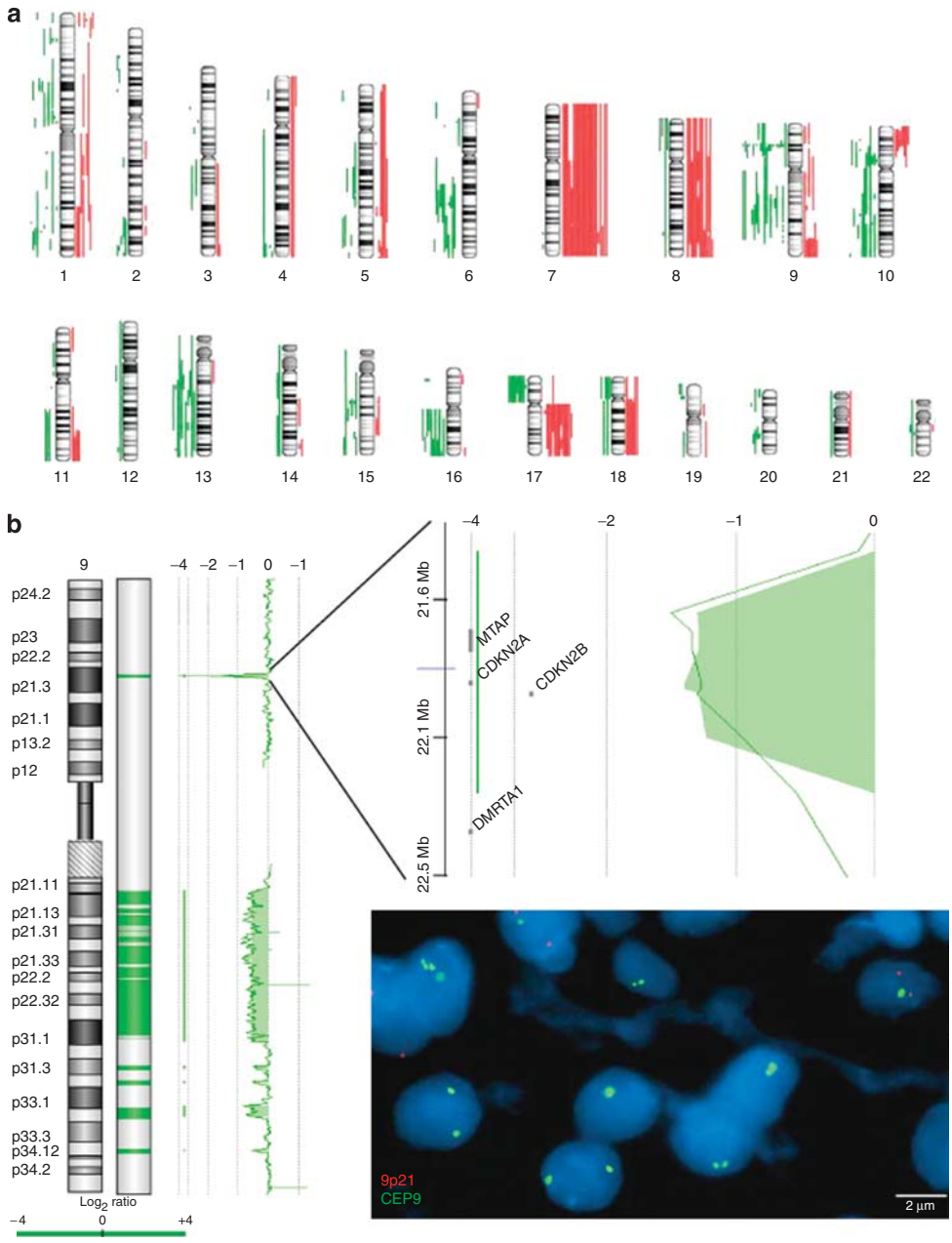
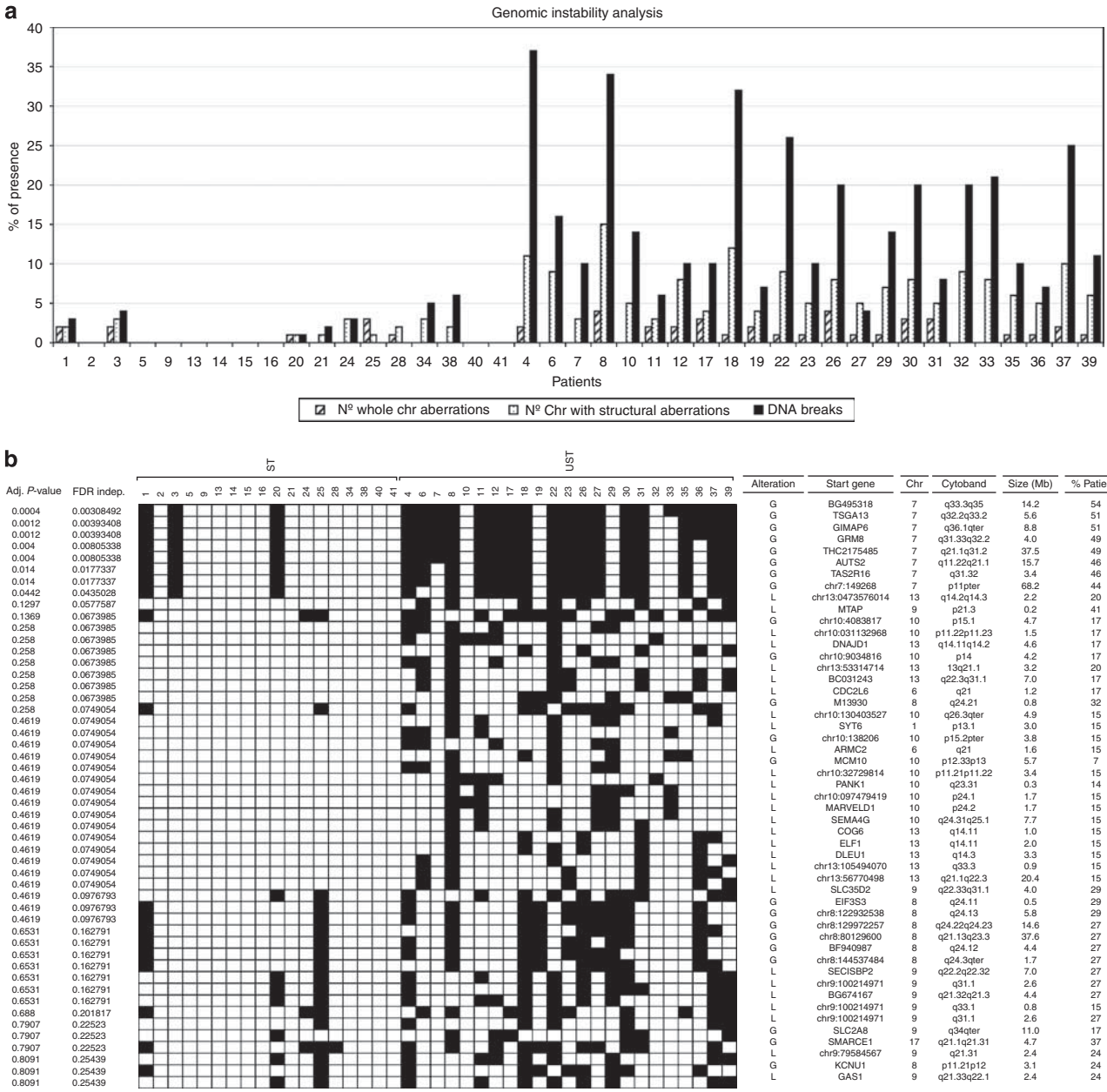


Figure 1. Oligonucleotide arrayCGH (comparative genomic hybridization) results and fluorescence *in situ* hybridization (FISH) validation. (**a**) ArrayCGH was applied in 41 tumor stage mycosis fungoides (MFt) frozen tissue samples and a total of 32 patients had an aberrant genomic profile. All the abnormalities found are represented in the idiogram: the red line at the right side represents gains, whereas the green line at the left side represents losses of genomic DNA. (**b**) Log_2 ratio values along the chromosome are represented by a green line. The vertical line around 0 corresponds to no copy number changes. Displacement of this green line to the left or right represents genomic losses or gains, respectively. To validate the arrayCGH results and to corroborate the gain and loss thresholds, the fluorescence *in situ* hybridization (FISH) technique was applied in the paraffin-embedded tissue sections of 3 patients. All FISH results were in concordance with those found earlier by arrayCGH.A homozygous deletion of 9p21.3 is shown here. Scale bar = 2 μm.

of 7q. The genomic instability profile of MFt is summarized in Figure 2. All parameters analyzed are provided in Supplementary Table S3.

Statistical analysis

The prognostic value of the genomic instability status (stable MFt *vs* unstable MFt), and specific altered chromosomal regions with a frequency higher than 15% of cases was analyzed. In the univariate analysis, the genomically unstable MFt group disclosed a worse prognosis (median overall survival, OS: 88 *vs* 43 months; $P = 0.05$). In addition, three specific chromosomal imbalances were associated with poor outcome: gains/amplifications of 8q24.21 (median OS: 89.1 *vs* 41 months; $P = 0.017$), as well as deletions of 9p21.3 (median OS: 85.5 *vs* 45.7 months; $P = 0.042$) and 10q26qter (median OS: 78 *vs* 19.7 months; $P = 0.023$) (Figure 3). Besides genomic aberrations, age older than 60 (median OS: 131.54 *vs* 47.5 months; $P = 0.007$) and multifocal



Alteration	Start gene	Chr	Cytoband	Size (Mb)	% Patients
G	BG495318	7	q33.3q35	14.2	54
G	TSGA13	7	q32.2q33.2	5.6	51
G	GIMAP6	7	q36.1qter	8.8	51
G	GRM8	7	q31.33q32.2	4.0	49
G	THC2175485	7	q21.1q31.2	37.5	49
G	AUTS2	7	q11.22q21.1	15.7	46
G	TAS2R16	7	q31.32	3.4	46
G	chr7:149268	7	p11pter	68.2	44
L	chr13:0473576014	13	q14.2q14.3	2.2	20
L	MTAP	9	p21.3	0.2	41
G	chr10:4083817	10	p15.1	4.7	17
L	chr10:031132968	10	p11.22p11.23	1.5	17
L	DNAJD1	13	q14.11q14.2	4.6	17
G	chr10:9034816	10	p14	4.2	17
L	chr13:53314714	13	13q21.1	3.2	20
L	BC031243	13	q22.3q31.1	7.0	17
L	CDC2L6	6	q21	1.2	17
G	M13930	8	q24.21	0.8	32
L	chr10:130403527	10	q26.3qter	4.9	15
L	SYT6	1	p13.1	3.0	15
G	chr10:138206	10	p15.2pter	3.8	15
L	ARMC2	6	q21	1.6	15
G	MCM10	10	p12.33p13	5.7	7
L	chr10:32729814	10	p11.21p11.22	3.4	15
L	PANK1	10	q23.31	0.3	14
L	chr10:097479419	10	p24.1	1.7	15
L	MARVELD1	10	p24.2	1.7	15
L	SEMA4G	10	q24.31q25.1	7.7	15
L	COG6	13	q14.11	1.0	15
L	ELF1	13	q14.11	2.0	15
L	DLEU1	13	q14.3	3.3	15
L	chr13:105494070	13	q33.3	0.9	15
L	chr13:56770498	13	q21.1q22.3	20.4	15
L	SLC35D2	9	q22.33q31.1	4.0	29
G	EIF3S3	8	q24.11	0.5	29
G	chr8:122932538	8	q24.13	5.8	29
G	chr8:129972257	8	q24.22q24.23	14.6	27
G	chr8:80129600	8	q21.13q23.3	37.6	27
G	BF940987	8	q24.12	4.4	27
G	chr8:144537484	8	q24.3qter	1.7	27
L	SECISBP2	9	q22.2q22.32	7.0	27
L	chr9:100214971	9	q31.1	2.6	27
L	BG674167	9	q21.32q21.3	4.4	27
L	chr9:100214971	9	q33.1	0.8	15
L	chr9:100214971	9	q31.1	2.6	27
G	SLC2A8	9	q34qter	11.0	17
G	SMARCE1	17	q21.1q21.31	4.7	37
L	chr9:79584567	9	q21.31	2.4	24
G	KCNU1	8	p11.21p12	3.1	24
L	GAS1	9	q21.33q22.1	2.4	24

Figure 2. Genomic instability profile analysis and multiple testing. (**a**) The quantitative assay for genomic instability was performed considering the DNA breaks (DBs)(■), whole chromosome abnormalities (◪), and the number of DBs within a chromosome (□). It clearly distinguished between the genomic stable subgroup on the left side of the graphic and the genomic unstable subgroup on the right side, which has a higher representation for all the analyzed parameters. (**b**) Multiple testing was performed with Pomelo Cluster Tool 2.0 to compare the relationship between the genomic status and the smallest overlapping region of imbalances found. A significant correlation between the genomic unstable tumor stage mycosis fungoides (MFt) group and the 7q regions was observed.

localization of cutaneous lesions (>2 presentation sites) (median OS: 82.6 *vs* 39.2 months; $P=0.033$) were associated with shorter survival. No association between extracutaneous involvement, presence of ulceration, cell size, epidermotropism, and survival was detected. The multivariate survival analysis, taking into account the parameters considered statistically significant by the univariate analysis, did not reveal any independent prognostic factor (Table 2). This fact could arise from the low number of patients. Despite this result, it is important to mention that this study is the largest series reported until now in MFt as it is a very infrequent disease.

DISCUSSION

We present here a DNA genomic imbalance detailed analysis of the largest series of MFt patients reported until now, including 41 patients. In addition, we describe their association with clinical data and prognosis.

Recently, van Doorn *et al.* (2009) have used a BACarrayCGH platform for the identification of genomic differences

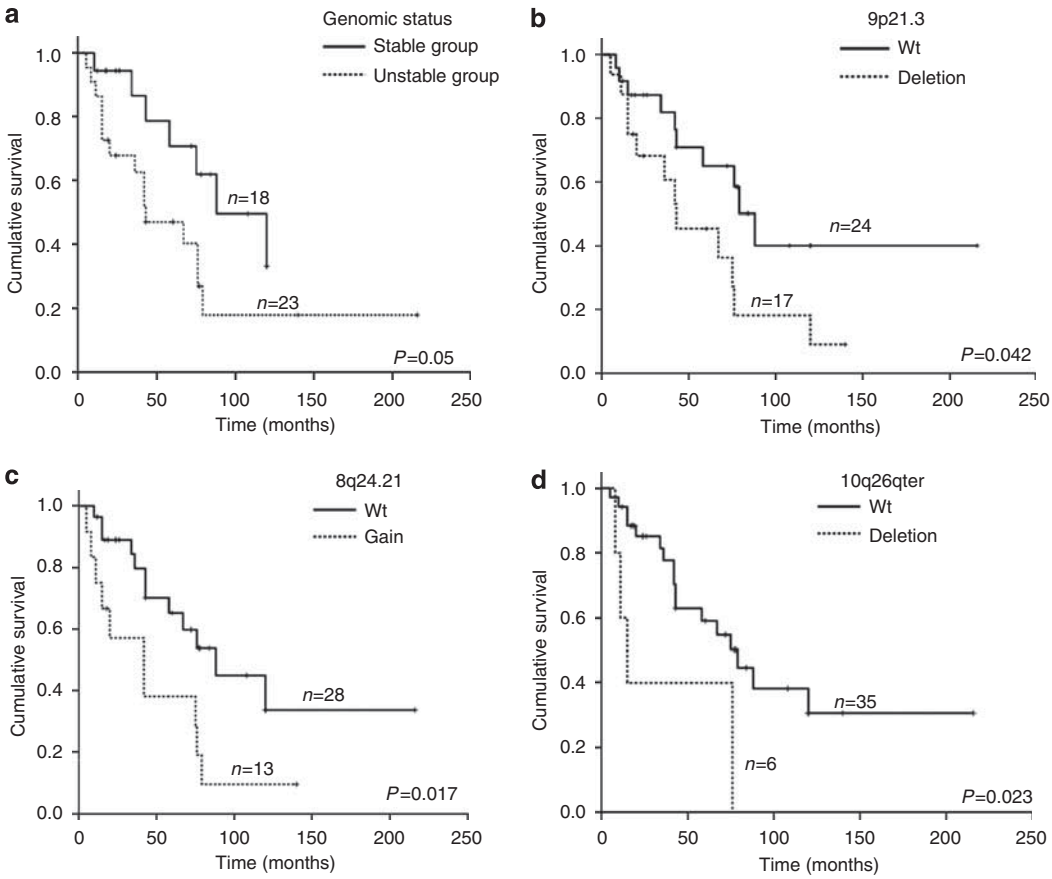


Figure 3. Impact of genomic imbalances and genomic instability groups on survival of tumor stage mycosis fungoides (MFt). A log-rank test was used to evaluate the correlation between the genomic profile and the survival of the MFt patients. (**a**) The Kaplan–Meier curves showed survival differences between genomic unstable MFt patients (dotted line) and genomic stable MFt patients. (**b**) Regarding specific lesions, a poor overall survival was observed in MFt patients with 9p21.3 deletion (dotted line), (**c**) gains of 8q24.21 (dotted line), and (**d**) 10q26qter deletion (dotted line) compared with MFt patients with no chromosomal aberrations.

Table 2. Results of the univariate and multivariate survival analysis

Variables (*n*=41)	Median OS (months)	Univariate analysis *P*-value	Multivariate analysis *P*-value
Genetic alterations			
del(9p21.3)	85 *vs* 46	0.04	NS
del(10q26qter)	78 *vs* 20	0.02	NS
+8q24.21	89 *vs* 41	0.02	NS
Genetic status			
Stable *vs* Unstable	88 *vs* 43	0.05	NS
Clinicopathological parameters			
Age (<60 *vs* ⩾60 years)	131 *vs* 47	0.01	NS
Cutaneous localization (localized *vs* multifocal)	83 *vs* 39	0.03	NS

NS, not significant.

between SS and MFt, describing a high frequency of gains in chromosomes 1, 7, 8, and 17 and losses of chromosomes 5, 9, and 13. This study has allowed the detection of small aberrations, with the smallest abnormality reported being 1.3 Mb in size. With the genomic platform used in this study, which includes about 44,000 probes covering the whole genome at an average resolution of 75 kb, a genome-wide analysis of a large series of MFt has been performed. Ten cases from van Doorn *et al.* (2009) analysis were also analyzed with oligonucleotide-based arrayCGH to compare both platforms. The vast majority of the aberrations were detected by both BACarrays and oligonucleotide-arrays. Although the detection of gains is very similar, it is important to emphasize that we found a higher number of losses and that they were characterized by their smaller size. The combination between oligo-arrays and the InSilico arrayCGH analysis has allowed us to delineate the MFt chromosomal alterations in more detail.

In terms of chromosomal imbalances, 78% ($n=32$) of our patients presented with an aberrant genomic profile. The high rate of genetically abnormal patients could be explained by the fact that all the patients have been studied at an advanced

stage and therefore the proportion of malignant T-cell lymphocytes is very high as described earlier (Mao *et al.*, 2003; Fischer *et al.*, 2004; Prochazkova *et al.*, 2007). The nine patients who did not present genomic abnormalities could present other altered genetic mechanisms, such as gene mutations, methylation, aberrant miRNA expression, or acquired uniparental disomy, which do not implicate gains or losses of DNA. Therefore, the exploration of this type of mechanism is necessary to elucidate other genetic alterations that can explain the biology of this tumor. Regarding specific alterations, losses were more frequently detected than gains (5.7 losses *vs* 3.3 gains), in contrast to the recent findings by Prochazkova *et al.* (2007). The analysis by Prochazkova *et al.* (2007) was performed with the conventional CGH technique (mean resolution: 5–10 Mb). With this technique, gains and losses smaller than 5 Mb were not detected. In contrast, the oligonucleotide-based array GCH platform used in this study allowed us to detect gains and losses bigger than 75–100 Kb. This could be one reason that explains the discrepancies between the most frequent gains and losses among the two studies. Moreover, the sample size analyzed in this study was bigger (41 patients *vs* 11 patients in Prochazkova *et al.* (2007) study). However, Prochazkova *et al.* (2007) provided additional information regarding the DNA content (mean DNA index 3.14 ± 0.38), which was not analyzed in this study.

Oligonucleotide arrayCGH analysis has allowed the description of MFt in the context of genomic instability. It has been suggested that failures in a number of different processes that maintain genome integrity could contribute to the wide variety of genomic alterations in solid tumors. These aberrations include the total gain or loss of whole chromosomes or parts of chromosomes, HLAs (defined as a copy number increase of a determined region of a chromosome), HDs (loss of the two copies of a specific region), and copy number transitions (number of DBs within a chromosome). Analyses of genomic instability have been reported for bladder cancer, breast tumors, neuroblastoma, B-cell lymphomas, and Ewing's tumor (Blaveri *et al.*, 2005; Fridlyand *et al.*, 2006; Ferreira *et al.*, 2008a, b). In such cases, a correlation between highly unstable genetic profile and poor prognosis has been shown. We have performed a quantitative analysis of the genomic instability in MFt patients taking into account the above mentioned parameters (Supplementary Table S3). We observed two different groups, one genomically stable MFt characterized by a low number of chromosomal abnormalities and the other genomically unstable showing a high number of chromosomal abnormalities. Moreover, the univariate survival analysis clearly showed that MF patients showing a genetic unstable pattern have a shorter survival ($P = 0.05$). Therefore, the rather consistent pattern of genomic abnormalities provides reliable information to understand the genetic bases that underlie the clinical phenotypes of MFt with different survival rates.

Regarding specific abnormalities, we detected two aberrations, 9p21.3 deletion and 8q24.21 gain, that correlate with poor prognosis, in agreement with recently published data in MFt patients (van Doorn *et al.*, 2009). Our findings confirm such results in a large series of patients and suggest the important implication of these two regions in the pathogenesis of MFt patients. Regarding 9p21.3 deletion, we have delineated a minimal region of only 200 kb comprising only three genes *CDKN2A, CDKN2B,* and *MTAP.* Unlike the BACarray platform used by van Doorn *et al.* (2009) who detected two contiguous 9p21-deleted regions of 2 Mb in size, the oligo-arrayCGH technology has allowed the definition in more detail of the 9p region and the genes enclosed in these loci. Among these three genes, *CDKN2A* and *CDKN2B* have been largely studied in MF. The most frequent alteration has been the hypermethylation, but not mutation, of loss of heterozygosity (Navas *et al.*, 2000, 2002). In this study, we have also observed a high frequency of HD, not observed until now. On the other hand, the *MTAP* gene has also been described as an important tumor suppressor gene in several cancers (Nobori *et al.*, 1996; Dreyling *et al.*, 1998; Christopher *et al.*, 2002; Subhi *et al.*, 2004; Marcé *et al.*, 2006; Worsham *et al.*, 2006; Mirebeau *et al.*, 2006) and is an essential enzyme for normal activity of the adenine and methionine synthesis. The loss of this gene is thought to be incidental because of its proximity to *CDKN2A* and *CDKN2B*. However, cells that lack *MTAP* depend on *de novo* AMP synthesis and exogenous methionine supply, and are expected to be sensitive to inhibitors of purine synthesis or methionine starvation. A better understanding of the contribution of the *MTAP* gene in all stages of MF could provide an impetus for exploration of these targets as therapeutic biomarkers in MF.

Regarding chromosome 8, partial or complete gains on 8q have been observed in earlier studies in patients with MFt and SS (Prochazkova *et al.*, 2007; Vermeer *et al.*, 2008; van Doorn *et al.*, 2009). In our study, we detected a high number of patients with altered chromosome 8 and delineated a minimal common region, 8q24.21, in 31.4% (13/41) in concordance with recent reports (van Doorn *et al.*, 2009). In addition, two patients presented with an HLA of this location involving the *MYC* oncogene. *MYC* is generally recognized as an important regulator of proliferation, growth, differentiation, and apoptosis (Meyer *et al.*, 2006; Vita and Henriksson, 2006). Interestingly, we have observed in our series a strong correlation of this abnormality with a poor outcome of patients ($P = 0.017$). The recent finding of gain of *MYC* in SS (Vermeer *et al.*, 2008) and MFt (van Doorn *et al.*, 2009) and the correlation with survival could suggest an important involvement of *MYC* in the progression of a subset of MFt patients.

Abnormalities of chromosome 10 have been described earlier in MFt and SS detected by G-banding cytogenetic studies, conventional CGH, and microsatellite markers (Limon *et al.*, 1995; Karenko *et al.*, 1997, 1999; Scarisbrick *et al.*, 2000, 2001; Mao *et al.*, 2002, 2003; Espinet *et al.*, 2004; Fischer *et al.*, 2004; Wain *et al.*, 2005; Prochazkova *et al.*, 2007). We have detected 10q26qter deletion, a minimal common region, which is to our knowledge not reported earlier. This anomaly of only 0.7 Mb in size harbors a total of 31 genes. Among them, it is important to mention the presence of two tumor suppressor genes: *MGMT* and *EBF3*. Concerning the *MGMT* gene, its methylation status has been studied earlier in cutaneous T-cell lymphoma (Gallardo

et al., 2004; Van Doorn *et al*., 2005). However, the presence of methylation in healthy control T-cell lymphocytes led to the preclusion of its use as a marker of malignancy. On the other hand, the recent description of *EBF3* as a tumor suppressor gene that induces cell cycle arrest and apoptosis (Zhao *et al*., 2006) led to suggest the implication of this gene in the pathogenesis of MFt patients. Our analysis has shown a strong correlation between disease progression and deletion of this region ($P=0.021$). Thus, the genes included in 10q26qter should be studied to understand their pathogenic role in MFt patients.

Regarding chromosome 12, an interesting region is 12q21 where *NAV3* is localized. *NAV3* deletions and translocations were described as frequent genetic anomalies in MF and SS (Karenko *et al*., 2005). In our study, only one patient presented with a deletion of this region because of the loss of the long arm of chromosome 12. Therefore, our results are in concordance with Marty *et al*. (2009) who recently described that *NAV3* deletions and translocations are rare events in cutaneous T-cell lymphoma. Moreover, a high frequency of 12q24.31 deletions (involving *BCL7a*, *SMAC/DIABLO*, and *RHOF* genes) has been reported in early stage MF patients (Carbone *et al*., 2008). In contrast to this report, we have detected a deletion in only one patient and the loss of the entire chromosome 12 in a second one. Therefore, the validation of this finding in a selected tumoral population of the early stage MF biopsies would be necessary to confirm this anomaly. Most probably, the pathogenic mechanism related to this region in advanced stage patients was the hypermethylation of the tumor suppressor gene *BCL7a*, as reported earlier in cutaneous T-cell lymphoma patients (Van Doorn *et al*., 2005), but not the deletion of this area.

Among all the alterations detected in this analysis, we have observed that some of them are very similar to SS (Vermeer *et al*., 2008), such as gains of 17q21.1 and 8q24.21 and losses of 17p13.1 and 10p11.2. Although we have detected these alterations in less proportion and the vast majority of alterations are quite different, it is important to remark that among the three patients who presented with blood involvement, all of them had a loss of 17p13.1 and gain of 17q21.1 , and one of them presented with a loss of 10p11.22 and the another one with a gain of 8q24.21. Our findings support that Sézary syndrome patients have a different genomic profile than do MFt patients (van Doorn *et al*., 2009). However, the presence of similar aberrations in MFt patients who present with blood involvement seems to indicate that both pathologies have a similar origin. More studies comparing these two groups of patients (SS *de novo vs* SS with an earlier MF) will provide additional information of these entities.

In summary, oligonucleotide-based arrayCGH analyses have clearly shown a high frequency of genetic imbalances and chromosomal abnormalities, not reported earlier to our knowledge in MFt, which provide a strictly genomic characterization of this entity. Moreover, we report the genomic profile of MFt patients in terms of genetic instability, which is to our knowledge not reported earlier, categorizing the patients into two MFt genomic subgroups: a stable group (0–5 DNA aberrations) and an unstable group (>5 DNA aberrations). Furthermore, the correlation of the genomic status, as well as the deletion of 9p21.3 and 10q26qter and gain of 8q24.21 with the outcome, offers the possibility of selecting these patients to precisely adjust their clinical management. The detection of these alterations with routine techniques such as FISH and/or multiplex ligation-dependent probe amplification during the follow-up could be used to closely monitor this group of patients to identify particular subsets presenting a more aggressive clinical evolution. Validation of such genomic features represents a reasonable next step for the definition of biological prognostic factors enabling the design of optimized risk-adapted treatment strategies.

MATERIALS AND METHODS

Patients

A total of 41 patients collected from centers collaborating in the European Organization for Research and Treatment of Cancer (EORTC) Cutaneous Lymphoma Group were included in the study. They comprised 22 males and 19 females with a mean age of 59 years (range, 17–84 years). All patients were diagnosed according to the World Health Organization (WHO)-EORTC classification for cutaneous lymphoma criteria (Willemze *et al*., 2005; Olsen *et al*., 2007). Clinical and follow-up data are summarized in Tables 2 and 3. Ten patients were earlier studied using a BACarrayCGH platform (van Doorn *et al*., 2009). The approval for the study was provided by the Comitè Ètic d'Investigació Clínica from l'Institut Municipal d'Assistència
Sanitària (CEIC-IMAS) and written informed consent was obtained from all patients, according to the Declaration of Helsinki Principles.

DNA extraction

To ensure the high quality of the DNA analyzed, $20 \times 10\,\mu m$ snap-frozen samples from tumoral MF lesions were included in the study. A hematoxylin-eosin staining of a frozen section from all cases was performed earlier to confirm the presence of at least 70% of tumor cells. DNA was isolated using a commercial kit, as described (DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany).

Array CGH

Genome-wide analysis of patient samples was conducted using the Human Genome CGH 44K microarrays (G4410B and G4426B) (Agilent Technologies, Palo Alto, CA). The hybridization process was performed according to the manufacturer's protocols. Commercial pools of healthy female DNA (Promega, Madison, WI) were used as controls. For extraction of raw data and visualization of results, Feature Extraction v.8.1 and CGH Analytics v3.2.25 softwares were used (Agilent Technologies). Data analysis and chromosome segmentation were performed with InSilico Array CGH software smoothing methods (Conde *et al*., 2007) included in GEPAS (http://gepas.bioinfo.cipf.es). This software provided a copy number value that allowed the establishment of cutoff values at 0.3 and −0.5 for considering gains and losses, respectively. For HLAs and HDs, the cutoff values were set at 0.6 and 1, respectively. Recurrent regions involved in genomic imbalances were defined as a sequence of at least five consecutive altered probes common to a set of array CGH profiles and the smallest overlapping region of imbalance as the

Table 3. Clinical characteristics of patients with tumor stage MF (MFt)

Characteristics	MFt patients
Total no. of patients	41
Age, years	
Median	63
Range	17–84
Sex	
Male	22
Female	19
Cutaneous lesions[1]	
Solitary	1
Localized	16
Multifocal	24
Not available	1
Initial therapy, no.	
SDT	24
Immunomodulators	5
Polychemotherapy	1
Combination of different treatments	10
Not available	1
Response to initial therapy, no.	
CR	10
PR	16
PD	7
Not available	6
Relapse	
Skin only	13
Systemic	3
Follow-up, months	
Median	43
Range	5–216
Status at last follow-up, no.	
No evidence of disease	3
Alive with disease	16
Died as a result of lymphoma	22

CR, complete response was defined as the clinical and histological (when possible) disappearance of all lesions; PD, progressive disease was defined as the appearance of new lesions representing 25% over pre-existing lesions, or infiltration of 25% or more of pre-existing lesions; PR, partial response was defined as a 50% or greater decrease in the number and size of pre-existing lesions; SDT, skin directed treatment.
[1]Cutaneous localization: solitary, solitary skin involvement; localized, multiple lesions limited to 1 body region of 2 contiguous body regions; Multifocal, multiple lesions involving two noncontiguous body regions.

minimal common region detected in at least two patients (Rouveirol *et al.*, 2006). Genomic aberrations in known copy number polymorphisms were not considered as alterations.

Moreover, we have applied a genomic stability assay to evaluate the genetic status of this type of tumor. Total gain or loss of whole chromosomes or parts of chromosomes, HLAs (defined as a copy number increase of a determined region of a chromosome), HDs, and copy number transitions (the number of DBs within a chromosome) were quantified (Supplementary Table S3). A multiple testing tool (Pomelo Cluster; http://pomelo.bioinfo.cnio.es) was used to compare the genomic MFt patient status with all smallest overlapping region of imbalances found applying Fisher's test.

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization was performed to confirm chromosomal abnormalities detected earlier by arrayCGH in those cases in which a paraffin-embedded tissue biopsy was available. The FISH probes used are summarized in Supplementary Table S4.

Statistical analysis

Overall survival was calculated as the time elapsed from the first date of diagnosis of MFs to death of the lymphoma or to last follow-up. The Kaplan–Meier method was used to estimate the distribution of OS. Differences in survival between groups were assessed using the log-rank test. Multivariate Cox proportional hazards regression was performed. The following clinical, morphological, and genetic parameters were evaluated to identify risk factors in a univariate analysis for OS: age (<60 years against >60 years), sex, localization of cutaneous lesions, extracutaneous involvement, response to therapy, presence of large cells, genomic instability status, and presence of recurrent genomic abnormalities (more than 15% of cases). For comparison of two groups, the Mann–Whitney *U*-test and Pearson χ^2-test were used. Statistical computations were performed using the SPSS v.15 software (SPSS, Chicago, IL). A *P*-value of $\leqslant 0.05$ was considered statistically significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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