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Summary

Genetic variants of *NLRP3* and *NLRP1* are known to modulate levels of pro-inflammatory cytokine Interleukin (IL)-1β. The purpose of this study was to investigate the association of *NLRP3/NLRP1* polymorphisms with susceptibility and clinical features of malignant melanoma in a Swedish case-control study. Common variants in *NLRP3*/*NLRP1* were investigated in sporadic malignant melanoma patients and healthy controls followed by analysis using logistic regression. *NLRP3* variant (rs35829419) was significantly more common in male patients than in controls (OR 2.22, CI 1.27-3.86). Upon stratification, significant association to nodular melanoma was observed (OR 2.89, CI 1.33-6.30) which intensified in male patients (OR 4.03, CI 1.40-11.59). The *NLRP1* variant (rs12150220) was significantly more common in fair skinned female patients (OR 1.85, CI 1.04 -3.33) and showed strong associations with nodular melanoma (OR 6.03, CI 1.33-25). Our data suggests that NLRP3/NLRP1 polymorphisms are associated with melanoma susceptibility; these findings warrant validation in other independent populations.

Significance

An essential role of the pro-inflammatory cytokine IL-1β in tumor invasiveness and angiogenesis has previously been demonstrated. NLRP3/NLRP1 inflammasomes are IL-1β producing platforms that regulate inflammation. We report an increased susceptibility to sporadic malignant melanoma in the presence of common polymorphisms in the inflammasome genes. A particularly strong association was observed to nodular melanoma. Genetic alterations in the inflammasome genes might through dysregulations in IL-1β production result in increased susceptibility to melanoma.

Key words:

Inflammasome, NLRP1, NLRP3, CIAS1, SNP, melanoma, IL-1β

Introduction

NACHT-LRRs (NLRs) are a group of cytosolic receptors that recognizes intracellular microbial products as well as metabolic stress (Schroder and Tschopp, 2010). The NLR family includes 22 members and has a key role in regulation of inflammation and apoptosis (Schroder and Tschopp, 2010). The NLRs, NLRP1 (also termed NALP1) and NLRP3 (NALP3 or Cryopyrin) upon activation bind to an adaptor protein ASC to form an inflammatory complex termed the inflammsome, which cleaves pro-interleukin (IL)-1β and pro-IL-18 to mature and biologically active IL-1β and IL-18 (Fig.1) (Martinon et al., 2002, Agostini et al., 2004). Another adaptor comprising a function-to-find (FIIND) and caspase-activation and recruitment domain (CARD), termed CARD-8 (TUCAN or Cardinal) (Pathan et al., 2001), is postulated to be a binding partner of NLRP3, whereas its binding is not required by NLRP1, which already contains a C-terminal FIIND-CARD domain.

Missense mutations in the *NLRP3* gene lead to familial periodic fevers, where symptoms arise from excess of IL-1β and accordingly, blockade of IL-1β effectively ameliorates the symptoms (Hoffman and Simon, 2009). More recently, a single nucleotide polymorphism (SNP) in *NLRP3* (rs35929419), in combination with *CARD-8* polymorphism (rs2043211) have been associated with chronic inflammatory conditions (Verma et al., 2008, Kastbom et al., 2008, Schoultz et al., 2009, Roberts et al., Roberts et al., 2011). The CARD-8 protein is additionally suggested to be a negative regulator of caspase-1-mediated IL-1β regulation and NF-κB activation (Razmara et al., 2002, Fontalba et al., 2008). Another SNP in *NLRP3* (rs10733113), located downstream of the *NLRP3* gene, has been shown to regulate NLRP3 expression and IL-1β levels, and has recently been implicated in Crohn's disease (Villani et al., 2009). *NLRP1* SNPs rs12150220 and rs6502867 have been reported to be associated with susceptibility to vitiligo (Jin et al., 2007b), an autoimmune disease affecting the melanocytes in the skin, where patients are reported to have elevated serum IL-1β levels (Tu et al., 2003).

Several recent reports describe the importance of inflammatory microenvironment in the risk of tumor initiation and progression (Coussens and Werb, 2002, Mantovani et al., 2008, Clevers, 2004, Coussens and Werb, 2001). Tumors secrete pro-inflammatory cytokines into the surroundings (Okamoto et al., 2010), which accelerate tumor development and progression in a number of ways e.g. by inducing proliferation and survival of malignant cells, by promoting angiogenesis and metastasis, and by altering responses to chemotherapeutic drugs (Mantovani et al., 2008). The molecular mechanisms linking inflammation and carcinogenesis, however, still remain to be clarified. Malignant melanoma (MM) originates from the pigment-producing melanocytes in epidermis and is the most severe type of skin cancer. Melanoma often displays an aggressive behavior with early metastases and its incidence increases faster than any other cancer type among fair skin population worldwide (Diepgen and Mahler, 2002, Lens and Dawes, 2004). The survival rate of melanoma patients is directly related to

early detection and treatment and hence the search for genetic markers of disease risk is of continuing medical interest.

The present study investigates the significance of five inflammasome-related SNPs; rs35829419 and rs10733113 in *NLRP3*, rs6502867 and rs12150220 in *NLRP1*, and rs2043211 in *CARD-8*, in susceptibility and progression of MM.

Material and methods

Study subjects

This study was approved by the Regional Ethics Committee at the Linköping University, Linköping, Sweden. 258 patients (53% females, 47% males, ≥18 years) diagnosed with sporadic MM in the skin were included in the study. The patients were recruited from dermatology clinics in Southeastern region of Sweden (Linköping, Norrköping, and Kalmar). The mean age at inclusion was 59 years (±14 years, range 23-87 years). A detailed description of the study subjects is listed in Table I. The mean age of the melanoma patients at the time of diagnosis was 55 years (±15 years, range 18-86 years). The skin type was classified according to Fitzpatrick (Fitzpatrick, 1988) and grouped in sun-sensitive (I and II) and less sun- sensitive (III and IV) skin types. The eye color of the melanoma patients was divided into two groups, blue/green and brown/mixed and hair color into blond/red and brown/black. Histopathological types of melanoma were in the following categories: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo malignant melanoma (LMM), acro-lentiginous melanoma (ALM), and melanoma *in situ*, and the Breslow thickness was grouped into < 2 and ≥ 2 mm. Tumor location was registered using a schematic body chart as previously described (Synnerstad et al., 2004) and was classified into sun exposed (D,G,I,K,L,M,N) and rarely/ or not-sun exposed (C,H,J) areas.

The control population comprised 792 healthy individuals (50% females, 50% males) with mean age of 54 years (±17 years, range 20-80 years), randomly collected from the population register from the same geographic region as the patients with sporadic MM (Table I).

Genotyping

Genomic DNA was isolated from blood using the DNA blood maxi kit from Qiagen (Hilden, Germany). The rs35829419 and rs10733113 in *NLRP3*, rs6502867 and rs12150220 in *NLRP1*, and rs2043211 in *CARD-8* were genotyped using TaqMan® SNP Genotyping assays (C_25648615_10, C_30713847_10, C_11708080_1, C_29222211_20, and C_1600653_10) respectively), according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA). All analyses were performed in an ABI Prism 7500 Sequence Detection System, using the SDS 2.3 software for allelic discrimination (Applied Biosystems).

Statistical analysis

The STATA v. 10 (Stata Corp., College Station, TX, USA) statistical package was used to analyze the results. A comparison between cases and controls was performed with logistic regression analysis. Results are expressed as odds ratio (OR) with a corresponding 95% confidence interval (CI). Logistic regression was also used for calculations of the interactions between genes by using linear combinations of the coefficients of the variables. The variables were included one by one, together with the cross product term. The combination of variables was calculated by adding the coefficients and translated into an OR.

Results

In the present case control study, the SNPs rs35829419 and rs10733113 in *NLRP3*, rs2043211 in *CARD-8*, and rs6502867 and rs12150220 in *NLRP1* were studied in 258 patients diagnosed with sporadic MM and 792 controls. Both cases and controls were from the Southern part of Sweden. The description of the study subjects and stratifications of phenotypic features and melanoma characteristics are detailed in Table I and the genotype distributions are presented in Table II. All the SNP frequencies were found to be in Hardy-Weinberg equilibrium.

NLRP3 and *CARD-8* polymorphisms in patients with sporadic MM

No association was detected between the *NLRP3* rs35829419 and the overall risk for developing MM (Table III). Owing to the small numbers (1% in cases as well as controls), AA was analyzed in combination with the CA genotype group. As we have previously observed gender-based differences in genotype frequencies of rs35829419 (unpublished observation), we stratified the patients with sporadic MM and their controls based on gender and found the presence of at least one A allele to be more frequent among male patients (20%) than in male controls (10%) (p=0.005, OR 2.22, CI 1.27-3.86) (Table III). No corresponding risk was detected among women.

Stratifying the patients by melanoma type, i.e. NM versus SSM, revealed a significantly increased risk for developing NM in the presence of least one A allele (p=0.007, OR 2.89, CI 1.33-6.30) (Table IV). Furthermore, upon stratifying the patient group by gender (Table IV), a higher frequency of the A allele was observed among the male patients (20%) as compared to the female patients (12%). This difference, however, did not reach statistical significance (p=0.069, OR 1.89, CI 0.95-3.76). Furthermore, an even higher risk was associated with the male NM patients (p =0.010, OR 4.03, CI 1.40-11.59). The other types of melanoma (ALM, LMM) were not analyzed due to their small numbers.

The additional SNP studied *in NLRP3*, rs10733113, revealed no associations even after stratifications for the clinical parameters (all p-values >0.05) (Table III). However, the logistic regression analysis demonstrated that the combined genotypes rs35829419 and rs10733113 (CA,AA/GA,AA) were significantly more frequent in subjects with sporadic MM than in controls (p=0.001, OR 2.93, CI 1.58-5.44) suggesting a synergistic effect.

No overall association was found between the *CARD-8* SNP rs2043211 and MM. Moreover, no significant association was found after stratification for gender and melanoma type.

Statistical analyses of the three polymorphisms in relation to skin type (I+II to III+IV), eye color (blue+green to brown+mixed), Breslow thickness (≥ 2 mm to < 2 mm), age at diagnosis (≤ 35, 36-60,

> 60 years), and anatomic tumor site (sun exposed to rarely/not sun exposed) were performed, however no associations were found.

NLRP1 polymorphisms in patients with sporadic MM

No significant overrepresentation of the *NLRP1* SNPs was found in patients with sporadic MM when comparing with the control population (Table II). An overrepresentation of the A allele (TA/AA genotype) in the rs12150220 was observed in the patient group, which did not reach statistical significance (p=0.06, OR 1.37, CI 0.99-1.89).

When stratifying according to skin type, a 1.85 fold increased risk for MM was seen for the A allele in the rs12150220 among individuals with the fair skin types (type I and II) (p=0.037, OR 1.85, CI 1.04-3.33) (Table V). This risk further increased to 3.06-fold when analyzed only in the female patients (p=0.049, CI 1.01-0.09). No association was observed in the respective group of males.

Upon stratification by melanoma type, no association of any of the *NLPR1* SNPs was found (Table V). However, when analyzing based on gender, a strongly increased risk for developing NM was found for the rs12150220A allele among females (p=0.020, OR 6.03, CI 1.33-25). This increased risk was not detected in males.

Statistical analyses of both the *NLRP1* polymorphisms in relation to eye color, Breslow thickness, age of diagnosis, and anatomic tumor site of the body were performed, without statistically significant differences.

Discussion

The current study has investigated the association of the SNP's rs35829419 and rs10733113 in *NLRP3*, rs2043211 in *CARD-8*, and rs6502867 and rs12150220 in *NLRP1* in patients with sporadic MM. NLRP3 is a quite recently described member of the NLR family that is an essential regulator of the innate immune responses. NLRP3 forms an inflammatory complex called the inflammasome, which activates caspase-1 that subsequently leads to the formation of the potent proinflammatory cytokines IL-1β and IL-18. Missense alterations in *NLRP3* are postulated to confer a gain of function resulting in uncontrolled production of IL-1β, as seen in the patients with hereditary periodic fevers (Aksentijevich et al., 2002). The rs35829419 (Q705K) in *NLRP3*, due to its prevalence in the healthy population was initially described as a low-penetrance polymorphism (Aksentijevich et al., 2007) but in recent years this SNP has been associated with chronic inflammatory conditions. These associations have especially been seen in combination with rs2043211 in *CARD-8* (Verma et al., 2008, Kastbom et al., 2008, Schoultz et al., 2009, Roberts et al., Roberts et al., 2011). Using genetic construct for rs35829419 in an *in vitro* functional model, we have recently shown this SNP to confer a gain of function in terms of increased spontaneous IL-1β production from a human monocyte cell line (Verma, 2012).

In this study we found an increased overall risk for sporadic MM susceptibility among Swedish males carrying rs35829419A in *NLRP3*. The combination of rs35829419A with rs2043211 displayed no further increase in susceptibility risk for MM. Notably, a strong association was observed between NM and *NLRP3* rs35829419. Unlike SSM which initially describes a radial growth pattern, NM arises rapidly and grows in a more vertical direction and might be associated with a worse prognosis (Pollack et al., 2011). We hypothesize that this gain of function SNP due to increased IL-1β levels, might upregulate other related pro-inflammatory genes like TNF-α and NF-κB through a positive feed-back loop, which might promote melanoma cells to resist elimination by the T lymphocytes. This survival phenomenon has previously been demonstrated in melanoma cell lines treated with cytokines like TNF-α (Englaro et al., 1999) and very recently IL-1β (Kholmanskikh et al., 2010).

The fact that the SNP rs35829419A constituted a susceptibility risk only in the male population has previously been observed by us in Crohn´s disease (Schoultz et al., 2009). It is tempting to speculate that this differential risk pattern might depend upon the differences in estrogen levels, since the estrogen receptor by acting as a co-factor for NF-κB can inhibit its signaling (Biswas et al., 2005). Moreover, estradiol augments IL-1β mediated pro-inflammatory response by downregulating the IL-1 receptor type-1, probably accounting for an increased tolerance level in females (Schaefer et al., 2005).

NLRP1 polymorphisms are strongly associated with increased predisposition to autoimmune diseases (Jin et al., 2007b, Jin et al., 2007a). We report an increased risk with the rs12150220 variant in the individuals with a fair skin. Intriguingly, unlike our previous observation, this SNP was found to constitute a risk only in the females. NLRP1 inflammasome activates caspase-5 in addition to caspase-1. Caspase-5 is an upstream activator of caspase-1 and hypothetically, these two caspases together would result in a more effective IL-1β processing. Jin and colleagues have reported several *NLRP1* variants (including rs6502867 and rs12150220), that confer susceptibility to autoimmune and autoinflammatory diseases that are associated with vitiligo (Jin et al., 2007b). The fact that the SNP rs121150220 (L155H) is highly conserved through primate evolution (human, chimpanzee, Rhesus Monkey and Bush Baby) (Jin et al., 2007b), indicates that this region is critical for the function of the NLRP1 protein. This region has also been implicated in NOD2-NLRP1 interaction (Wagner et al., 2009). Functional studies elucidating the role of rs12150220 are lacking but it is tempting to speculate that this SNP leads to an overactivation of the inflammasome and hence contributes to melanoma predisposition.

This is the first study, to our knowledge, showing inflammasome polymorphisms to be associated with tumor susceptibility and melanoma type. Many cancers are known to arise from sites with inflammation (Mantovani et al., 2008). In addition, IL-1β is also detected in various tumor types (Jin et al., 1997, Culig et al., 1998, Oelmann et al., 1997, Saijo et al., 2002), where its expression is associated with tumor invasiveness and angiogenesis (Voronov et al., 2003, Carmi et al., 2009), suggesting a possible role for the inflammasome in tumorgenesis. In line with this, late stage human melanoma cells were demonstrated to spontaneously secrete active IL-1β via constitutive activation of the NLRP3 inflammasome in the absence of exogenous stimulation, exhibiting a feature of autoinflammatory diseases (Okamoto et al., 2010). We hence postulated that the gain of function SNP due to increased IL-1β production might contribute towards increased melanoma susceptibility. In a recent study using a mouse melanoma model it was demonstrated that inflammasomes contributed towards inhibition of the endogenous antitumor response and facilitated tumor growth (van Deventer et al., 2010). Further, in the highly invasive melanoma cells the expression of matrix metalloproteinase-1 (MMP), which is essential for tumor invasion and metastasis, is seen to be increased in an IL-1α and FGF2 dependent manner (Loffek et al., 2005). Notably, both IL-1α and FGF2 are regulated by caspase-1 activity (Keller et al., 2008), providing further support for the role of inflammasomes in melanoma. IL-18, another cytokine associated with inflammasome has been shown to be highly expressed in malignant skin tumors (Park et al., 2001), further emphasizing the role of inflammasomes in tumorgenesis.

Additional support for a critical role of IL-1β in the tumor microenvironment is evident by the success of IL-1 blockade in preventing angiogenesis and metastasis in animal models (Chirivi et al., 1993,

Vidal-Vanaclocha et al., 1994, Vidal-Vanaclocha et al., 1996, Carmi et al., 2009) as well as in human melanoma cell lines (Okamoto et al., 2010, Chirivi et al., 1993). A recent paper demonstrated increased IL-1β expression in primary and metastatic human melanomas and a decrease in melanoma cell growth was observed upon blocking the IL-1 signaling pathway (Qin et al., 2011). A potential role of IL-1β and inflammasomes in the pathogenesis of melanoma has recently been postulated (Zitvogel et al., 2012, Dunn et al., 2012). Above reports suggests that targeting IL-1 in tumors might indeed be a promising therapeutic option.

One caveat of the present study is the small number of patients resulting from sub-group analyses. However, we believe that the consistent trends in our results allow for the conclusion that NLRP3/NLRP1 polymorphisms influence susceptibility to MM and the development of NM.

In conclusion, we report inflammasome polymorphisms to be one link between innate immunity and susceptibility to sporadic MM. Further investigations are needed to confirm this finding on additional cohorts and elucidation of the importance of NLRP proteins in the pathogenesis of melanoma and other cancer types are warranted.

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Tables

Table I.

Patients with sporadic malignant melanoma and controls		
	Patients	**Controls**
Total number	258	792
Sex; males	120 (47%)	396 (50%)
females	138 (53%)	396 (50%)
Mean age	59±14	54±17
Mean age at diagnosis	55±15	-
Phenotype features of the patients and melanoma characteristics		
		Number
Age at diagnosis		
≤35		27 (11%)
36-60		129 (54%)
>60		84 (35%)
Skin type*		
I+II		153 (60%)
III+IV		100 (40%)
Eye color		
Blue + green		211 (82%)
Brown + mixed		46 (18%)
Hair color		
Blond + red		216 (84%)
Brown + Black		40 (16%)
Histopathological type		
Superficial spreading melanoma (SSM)		150 (64%)
Nodular melanoma (NM)		53 (22%)
Lentigo malignant melanoma (LMM)		9 (4%)
Acro-lentiginous melanoma (ALM)		2 (1%)
Melanoma *in situ*		22 (9%)
Breslow thickness (mm)		
<2		166 (77%)
≥2		50 (23%)
Anatomic site		
Sun exposed areas		176 (72%)
Rarely/ not-sun exposed areas		37 (15%)
Other areas		33 (13%)

*Skin type I+11, Sun-sensitive skin type, Skin type III +IV, Less sun-sensitive skin type

Table II. Distribution of genotypes.

	Sporadic MM	Controls
NLRP3 rs 35829419	257	792
CC	218 (85%)	692 (87%)
CA	36 (14%)	94 (12%)
AA	3 (1%)	6 (1%)
NLRP3 rs 10733113	257	792
GG	181 (71%)	574 (72%)
GA	70 (27%)	204 (26%)
AA	6 (2%)	14 (2%)
NLRP1 rs 6502867	255	784
TT	169 (66%)	497 (63%)
TC	80 (31%)	248 (32%)
CC	6 (3%)	39 (5%)
NLRP1 rs 12150220	254	790
TT	66 (26%)	157 (20%)
TA	132 (52%)	410 (52%)
AA	56 (22%)	223 (28%)
CARD-8 rs 2043211	257	792
TT	114 (44%)	330 (42%)
TA	111 (43%)	359 (45%)
AA	32 (13%)	103 (13%)

Table III. NLRP3 rs35829419 and NLRP3 rs10733113 genotype distributions in patients with sporadic MM. Genotype distributions for the two SNPs are also represented after gender stratification and in combination.

Genotypes	Sporadic MM	Controls	OR (95% CI)	P value
NLRP3 rs35829419	257	792		
CC	218 (85%)	692 (87%)	1	
CA/AA	39 (15%)	100 (13%)	1.27 (0.86-1.88)	0.22
NLRP3 rs35829419				
males				
CC	96 (80%)	355 (90%)	1	
CA/AA	24 (20%)	40 (10%)	2.22 (1.27-3.86)	**0.005**
females				
CC	121 (88%)	337 (85%)	1	
CA/AA	16 (12%)	60 (15%)	0.74 (0.41-1.34)	0.32
NLRP3 rs10733113	257	792		
GG	181 (70%)	574 (72%)	1	
GA/AA	76 (30%)	218 (28%)	1.13 (0.83 – 1.53)	0.44
NLRP3 rs10733113				
males	120	395		
CC	87 (73%)	287 (73%)	1	
CA/AA	33 (27%)	108 (27%)	1.01 (0.64 – 1.59)	0.97
females	137	397		
CC	94 (69%)	287 (72%)	1	
CA/AA	43 (31%)	110 (28%)	1.19 (0.78 – 1.82)	0.41
NLRP3 rs 35829419/rs 10733113				
CC/GG	169 (92%)	496 (87%)	1	
CA +AA/GA+AA	22 (8%)	22 (13%)	2.93 (1.58 - 5.44)	**0.001**

Table IV. NLRP3 rs35829419 genotype distribution in patients with sporadic MM upon stratifying for gender and melanoma type.

Stratification	Sporadic MM	OR (95% CI)	P value
gender	257		
males/females			
CC	96 (80%) /121 (88%)	1	
CA/AA	24 (20%) / 16 (12%)	1.89 (0.95-3.76)	0.069
Melanoma type	203		
***NM/SSM**			
CC	38 (72%)/132 (88%)	1	
CA/AA	15 (28%)/18 (12%)	2.89 (1.33-6.28)	**0.007**
gender/melanoma type	203		
males			
NM/SSM			
CC	16 (62%)/58 (87%)	1	
CA/AA	10 (38%)/9 (13%)	4.03 (1.40-11.59)	**0.010**
females			
NM/SSM			
CC	22 (81%)/ 74 (89%)	1	
CA/AA	5 (19%)/ 9 (11%)	1.87 (0.57 -6.16)	0.30

***NM, Nodular melamona; SSM, Superficial spreading melanoma

Table V. NLRP1 genotype frequencies in patients with sporadic melanoma and NLRP1 rs 12150220 genotype distribution after stratifying for gender, skin type and melanoma type.

Genotypes	**Sporadic MM**	**Controls**	**OR (95% CI)**	**P value**
NLRP1 rs 6502867	255	784		
TT	169 (66%)	497 (63%)	1	
TT/CC	86 (34%)	287 (37%)	1.18 (0.78-1-59)	0.27
NLRP1 rs 12150220	254	790		
TT	66 (26%)	157 (20%)	1	
TA/AA	188 (74%)	633 (80%)	1.37 (0.99-1.89)	**0.06**
Stratification	**Sporadic MM**	**OR (95% CI)**	**P value**	
Skin type*				
I+II/III+IV	157/97			
TT	32 (21%)/ 32 (33%)	1		
TA/AA	120 (79%)/65 (67%)	1.85 (1.04-3.33)	**0.037**	
Gender/skin type				
Females	86/45			
I+II/III+IV				
TT	20 (23%)/16 (36%)	1		
TA/AA	66 (77%)/ 29 (64%)	3.06 (1.01-9.09)	**0.049**	
Males	66/52			
I+II/III+IV				
TT	12 (18%)/16 (31%)	1		
TA/AA	54 (82%)/ 36 (69%)	2.00 (0.85-4.72)	**0.11**	
Gender /melanoma type				
Females	27/83			
#NM/SSM				
TT	2 (7%)/ 27 (33%)	1		
TA	17 (63%)/ 40 (48%)	5.74 (1.22.27)	**0.027**	
AA	8 (30%)/16 (19%)	6.75 (1.27-35.7)	**0.025**	
TA+AA	25 (93%)/ 56 (67%)	6.03 (1.33-25)	**0.025**	
Males	26/66			
NM/SSM				
TT	9 (35%)/13 (20%)	1		
TA	10 (38%)/ 35 (53%)	0.41 (0.14-1.24)	0.12	
AA	7 (27%)/18 (27%)	0.56 (0.17-1.90)	0.35	
TA+AA	17 (65%)/53% (80%)	0.46 (0.17-1.27)	0.14	

*Skin type I+II, Sun-sensitive; III+IV, Less sun-sensitive.
#NM, Nodular melanoma; SSM, Superficial spreading melanoma

Figure 1.

A simplified picture of IL-1β and IL-18 processing by the NLRP1 and NLRP3 inflammasomes. Upon sensing a stimuli, NLRP1/NLRP3 recruits the adaptor protein ASC and thereby activates caspase1 (and caspase 5 in case of NLRP1), which cleaves pro IL-1β and pro IL-18 to form the mature IL-1β and IL-18. These pro-inflammatory cytokines are then secreted out of the cell and upregulate a number of pro-inflammatory genes through a positive feedback loop. CARD-8 is postulated to be a binding partner of NLRP3, whereas its binding is not required by NLRP1 which already contains a CARD-8 domain.