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FAMILIAL MELANOMA IN SICILIAN PATIENTS

RESULTS OF SCREENING OF CDKN2A GENE MUTATION

PHds

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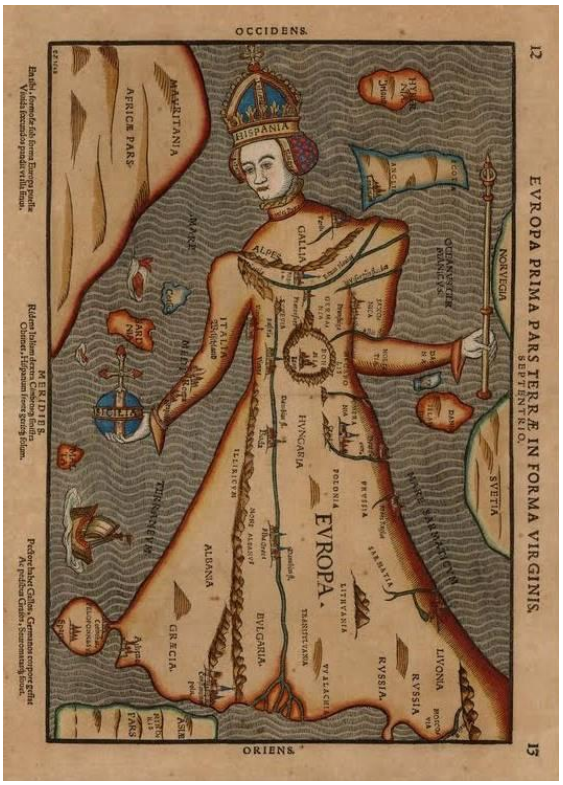
my loves

Virginia, Antonio, Bartolo

and to my beloved Sicily



The results of the genetic test seem to confirm the genetic diversity of Sicily (shown in the picture on the right hand of the Europe) compared to the rest of Europe and Italy. The high genetic heterozygosity, as shown by different anthropological-genetic studies, can be likely due to the history of Sicily that has always been, for its geographical location, a point of passage and meeting place of different Mediterranean people. Sicily was, during its history, the theater of invasions and dominations with genetic and cultural influences of different populations. In the east of Sicily it is still possible to identify genetic influences arising from the Greek domination, while in the west there are signs of the presence of the Phoenicians, the Normans and Arabs. (From Molecular analysis of the populations of the Mediterranean through 11 Alu insertions.Carla Maria Calò et al. 2005. Antropo, 9, 1-12. www.didac.ehu.es/antropo).

"As to the remote and exciting causes of melanosis, we are quite in the dark, nor can more be said of the methodus medendi. We are hence forced to confess the incompetency of our knowledge of the disease under consideration, and to leave to future investigators the merit of revealing the laws which govern its origin and progress….and pointing out the means by which its ravages may be prevented or repressed" –

Thomas Fawdington, The Manchester Royal Infirmary, 1826.

When Thomas Fawdington wrote these words in 1826, the medical science had little to offer to people with melanoma. The concept of inheritance and knowledge of DNA did not exist and had not yet realized that tumors had originated from the transformation of normal cells. Between 1650-1760 the European medical literature reported "fatal metastatic blacks tumors ... black liquid in the body ...", but one of the first and more detailed reports on the etiology and progression of melanoma was written by William Norris (1820), an english doctor (Stourbridge).

William Norris describes a 59 years-old patient with melanoma, describing the clinical evolution of the disease in a period of three years and, finally, describing the autopsy of the patient.

He described the macroscopic aspect of the primary lesion and similar lesions found in other organs. Based on his clinical observations and its anatomical findings he suggested that this disease had a high tendency to affect many organs. It 'was the first to suggest a relationship between nevi and melanoma and a possible link between the onset of melanoma and exposure to environmental factors (such as industrial pollution).

He described pigmented melanomas and amelanic lesions emphasizing the tendency of these lesions to metastatic spread to major organ systems. Norris also noted that no surgical or medical treatment was effective, once the disease was already metastatic. He argued, pioneer of surgery of melanoma, that only a wide excision of melanoma could control the locoregional recurrence.

Most importantly however, (and this is the reason why I started my thesis with a historical introduction to the disease) is that Norris was the first to notice the hereditary nature of some forms of melanomas, 50 years before Mendel presented his studies on the inheritance.

Norris pointed out in his report that the patient's father had died of a similar disease (to his son) that had probably originated from a nevus. The observation that the children of the patient had, like their father and grandfather, many nevi, pale skin and fair hair had led him to believe that there was some inheritance in the transmission of the tumor; Norris was probably the first to describe a case of melanoma Family.

One of the most significant advances in the understanding of melanogenesis was the discovery that tumors occur for genetic mutations of normal cells (1911, Peyton Rous and His studies on sarcoma chicken). One of the most significant advances in the understanding of melanogenesis was the discovery that tumors occur for genetic mutations of normal cells (1911, Peyton Rous and His studies on sarcoma chicken). In the early 80s the RAS oncogene family were discovered and began the "Oncogene Revolution" that changed the understanding of cancer and melanoma biology of the XXI century.

Norris studies were neglected by the scientific world until 1952, when Cawley describing a case of familial melanoma reopened the interest in this disease. Through a review of the scientific literature that included studies on animal models of melanoma (fish, reptiles, rodents, horses) he noted that even in animal species there was a mechanism of hereditary transmission of melanoma. The cases described were too few and it was not possible to assume a Mendelian mechanism of transmission.

The first case of identical twins with melanoma appeared at a young age was written in 1970. In the same years, a more systematic approach was that of Anderson that reviewing the medical records of 2,164 patients with melanoma treated at the Anderson

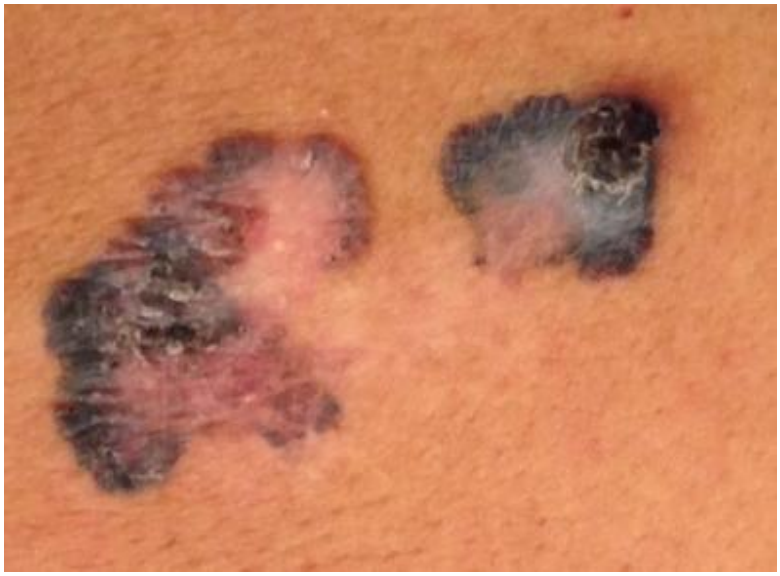
Hospital, identified 36 familiar forms and suggested, from the analysis of genealogical trees, that the hereditary melanoma was probably autosomic dominant, but more complex and multifactorial.

These assumptions are based on the observation that not all the children of the proband had or would have had a melanoma in their lifetime. He suggested that there had to be something (also an external factor) favoring the phenotype (the appearance of the tumor melanoma) in patients who were carriers of the dominant mutation gene. This "something" was what today is referred to as secondary factor responsible for LOH, loss of heterozygosity in the germline mutation, according to the hypothesis of Knusdon). (Greene et Bale, 1986). Even Wallac, 1973, failed to determine whether the transmission mechanism was polygenic or autosomal dominant with variable penetrance. (Wallace et al, 1973)

In those years there were studies on patients with dysplastic nevi and melanoma and the identification of hereditary dysplastic nevus syndrome (syndrome BK, DNS, FAMMM). Patients with familial melanoma not always had dysplastic nevi, and this led to a further nosographic characterization of the disease.

In 1956 Henry Lancaster, an Australian mathematician, who noted the increased incidence of melanoma in Caucasians living in Australia, underlined the probable etiopathogenetic role of UV radiation in the pathogenesis of the disease. Nelson later found a correlation between skin type and cutaneous melanoma, noting that those with pale skin type, migrated in geographical areas such as Australia and New Zealand (Celtic origin), were more likely to develop melanoma.

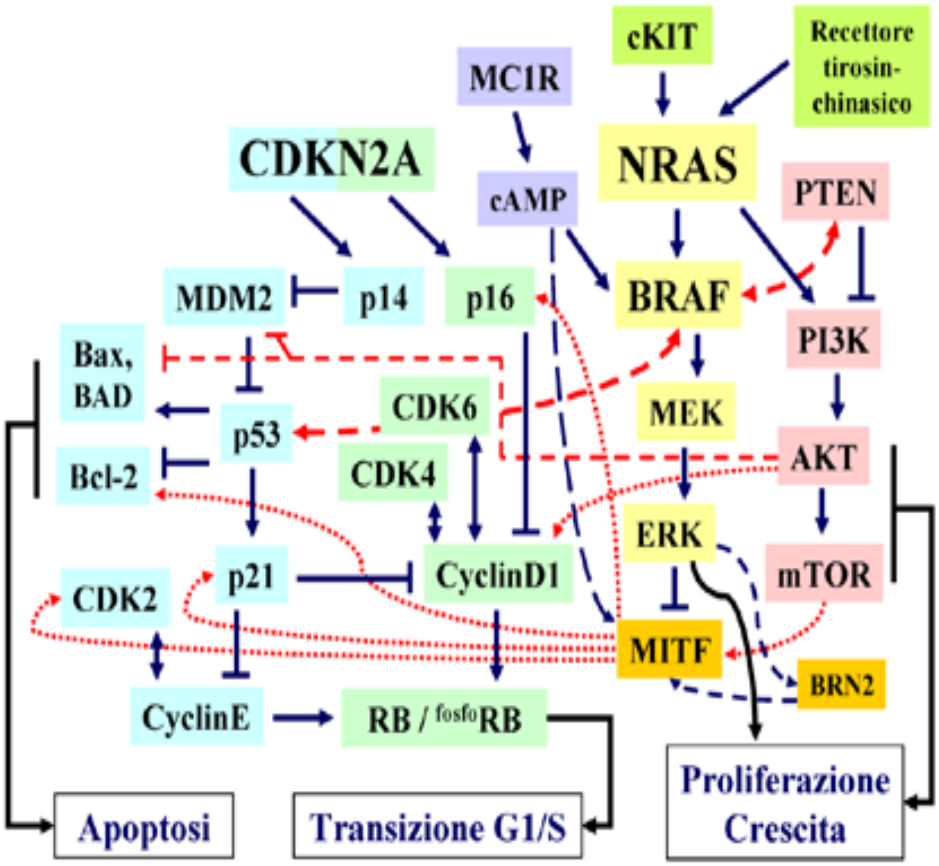
Then there were the pioneering studies of Clark (1966) and Breslow (1970) that classified melanoma according to the level of tumor infiltration and the staging of melanoma, according to their histopathological prognostic criteria, was standardized and is still the basis of classification system of the AJCC staging system (Rebecca W. et al. 2012).



Melanoma is a complex and heterogeneous disease. Genetic factors, individual and environmental factors are involved in its pathogenesis. The increased incidence of melanoma in the western world has carried this tumor to the attention of cancer research.

In recent years, it has been shown that melanoma is characterized by a strong molecular heterogeneity, considerably higher than that histological.

The pathogenesis of melanoma, as all other forms of malignant tumors, is due to the acquisition of sequential alterations of specific regions of DNA and / or alterations of particular mechanisms involved in the regulation of cell function; biomolecular allowed to characterize tumorigenesis as a process of accumulation of specific mutations that affect specific metabolic and molecular pathways (Held M, et al, 2010).



Major pathways involved in the pathogenesis of melanoma. From www.iss.it, scientific basis for the guidelines of the Ministry of Health.

Cytogenetic studies have identified mutations in genes involved in pathways regulating cell cycle as NRAS-BRAF (MAPK and PI3K-AKT pathway), CKIT and MITF and CDKN2A (p16CDKN2A-CDK4-RB and p14CDKN2A MDM2-p53 pathway) (Swick JM et al. 2012).

Subsequent studies correlated the type of genetic mutation with clinico-pathological variant of melanoma, showing a large heterogeneity between melanomas. These findings led the researchers to develop a molecular classification of melanoma, which today could allow, in advanced stages patients, to individualize therapies by identifying subgroups of patients who could benefit from a specific targeted therapy rather than the other. This is the reason for the increased interest today for the study of familial melanoma.

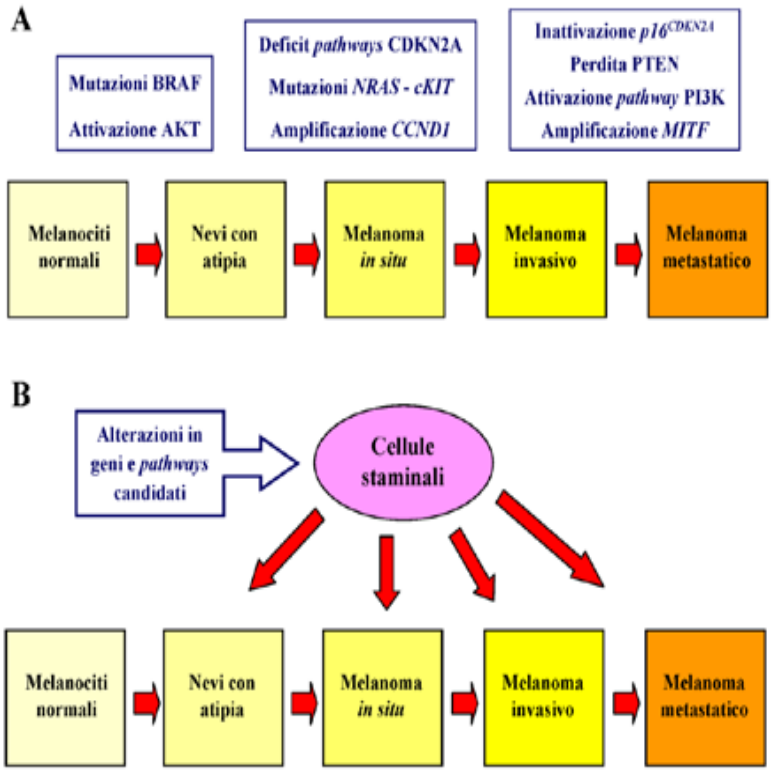
The overview of current information on the different pathways involved indicates the existence of complex molecular mechanisms of regulation, which shall ensure the integrity and regularity of different cellular functions in normal melanocytes. The progression from normal melanocytes in cancer melanocytes, up to aggressive and metastatic melanocytes, is therefore the result of events that increase or reduce the activity of various molecular pathways.

Recently it has been developed a model of “linear melanomagenesis” that is based on the accumulation of progressive molecular alterations.

It has also been hypothesized the existence, in some cases, not yet quantified, a second way of neoplastic transformation ("non-linear"), with the involvement of tissutal stem cells, whose alterations would give rise to direct melanoma cells in vertical growth phase or, even, in metastatic phase. This second hypothesis is based on evidence of some inconsistencies of progressive tumorigenesis in some subgroups of vertical growth melanomas than those in surperficial growth, as well as observation of amplification of genomic regions 9p21 and 1p22 prevalent in nodular melanomas. (Zabierowski SE, 2008; Fukunaga-Kalabis M et al, 2011;)

This aspect, in my opinion is very important, especially in familial melanomas in which it is not identified the mutation of the high penetrance gene CDKN2A. In this subgroup of patients, mutations of other genes (or alterations of other molecular pathways) could be transmitted in the hereditary line and induce the onset of melanoma in carriers.

According to the model of “non-linear” melanomagenesis a mutation can cause the onset of a superficial growth melanoma while another mutation could induce d’emblée a nodular melanoma or metastatic melanoma. The identification of the molecular mechanisms related to specific biological behavior and histological features, within of melanoma family would have tremendous value, enabling the identification of subtypes of melanomas with significantly different prognosis that may benefit from target and individualized specific therapy, not even in advanced stages of desease. (chemioprevention?).



Models of development and progression of melanoma. A model of sequential melanomagenesi. B non-linear model. (da www.isst.it)

These pathogenetic hypothesis and the noticeable molecular heterogeneity suggest different subtypes of melanoma, with different biological behavior and should lead to forget to the concept of the melanoma, from the biological point of view, as a single neoplasm.

The molecular classification of melanoma is destined to become increasingly complex and totally displace the more restrictive histo-cyto-patologic classification.

Mutation gene	Proportion of melanomas with mutations	phenotypic associations
BRAF	50%	young age intermittent sun exposure trunk and extremities high number of melanocytic nevi few freckles easily to tan histotype SSM
NRAS	15-20%	intermittent sun exposure (weak association) absent or poor pagetoid dissemination
c-Kit	2% (10-20% of acral and mucosal melanoma)	acral and mucosal melanoma
GNAQ/GNA11	40-50% uveal melanoma (Melanoma blue-nevus like?)	Uveal Melanoma

Table from www.iss.it of Ministero della Salute

The study of the correlation between type of genetic mutation and histopathological features of melanoma (Viros et al, 2008) showed that:

- Melanomas that arise in the trunk or in the anatomical sites intermittently

exposed to sunlight (or UV) are mostly SSM and have BRAF mutations. (J. Bauer 2011)

- - Melanomas arising on skin with solar elastosis, or signs of chronic exposure to sunlight (like arms, head and neck) have mutations of c-Kit and Nras genes and, only a few, of the BRAF. (J. Bauer et al 2011; Limon J. et al, 2013)

- Nodular melanomas do not have specific genetic mutations, supporting the hypothesis that nodular melanoma have a different origin and is not similar to other histological types of melanoma. (Curtin JA. Et al, 2005; 2006)

In the targeted therapy era, it is therefore essential to understand the complex biological mechanisms that underlie the development and progression of melanoma, to have a molecular classification of cancer and submit the patient to the most appropriate therapy for their own type of genetic-molecular aberration.

In the world, cutaneous melanoma is the 16th most common cancer in men and the 17th in women. In the last 40 years, the incidence of melanoma has increased, especially among the Caucasian population. In central European countries, the incidence rates increased from 3- 4 cases per 100,000 to 10-15 cases per 100,000.

The study of trends of incidence in Western countries indicates that incidence rates continue to increase even in the next two decades, and has led to talk of "melanoma epidemic". Mortality rates appear not to increase reflecting a likely improvement in early diagnosis. (Aiom Guidelines, 2013).

The reasons for this increased incidence is not yet well known. Changes in lifestyle and environmental factors (ozone hole?) seem to be the main responsible for this increased incidence of melanomas.

Risk factors for the development of cutaneous melanoma are both environmental and individual factors, related to the specific characteristics of the person.

Exposition to:
UV rays (sunburns)
UVB/UVA Rays (UV sunbeds)
ionizing radiations
PCBS
metallic dust
Arsenic
Heavy metals (lithographs)
cosmic radiation

Among the exogenous factors-environmental, sun exposure is determining factor in the etiology of melanoma (Jhappan et al, 2003), as confirmed by the data related to the high incidence rates in tropical geographical areas inhabited by populations of Northern Europe, with pale skin, or in evolutionary terms, unsuitable for that type of climate

There is a clear correlation between melanoma and white population, with blue eyes and blond hair, between the period of life spent in low latitudes and higher incidence of melanoma, between melanoma risk and intense, intermittent sun exposure, typically of summer recreation

Among other environmental factors potentially responsible for the onset of melanoma are: exposure to organic solvents (Lunderber, 1993; Langard, 2000; Loomis et al. 1997), ionizing radiation (Wennborg, 2001; Ron, 1998; Sigurson 2003; Tellelamberton 2004; Son E. 2001), PCBS, cosmic radiation (Haldorsen T. et al 2001; Pukkala 2002, 2003), arsenic (X. Guo et al, 2006; Y. Chen et al, 2007), heavy metals (Sarna et al, 1980), metal

powders (lithographs), (R. Dubrow et al 1986). In Italy has been demonstrated a significant association between occupational and environmental exposure to PCP, produced by Caffaro, Brescia, closed in the 80 Epidemiological data show an tripled incidence of melanoma in the last 15 years and case control studies showed a clear dose-response relationship.

Endogenous risk factors and Hereditary Melanoma

In most cases melanoma is sporadic, it occurs in subjects likely exposed to environmental risk factors such as UV rays. In a small percentage of cases melanoma is hereditary and occurs in individuals of the same family:

- - Carrier of common genetic variants associated with a slightly increased (compared to the population) incidence of melanoma or [
- in families with specific genetic mutations responsible for the onset of various diseases, oncologic and not, or
- - In families with specific genetic mutations that cause only the onset of melanoma (familiar melanoma proper) (C Daniell et al, 2011)

endogenous / individual Risk Factors
Ethnic group (Caucasian)
Pale skin type (I and III according to Fitzpatrick)
Conditions of immunosuppression (leukemia, drugs, etc)
Presence of several common nevi (> 50) or atypical / dysplastic,
dysplastic nevus syndrome
FAMMM (familial Atypical Multiple-Mole Melanoma Syndrome)
xeroderma pigmentosum
Albinism
Hereditary-family Neoplasms not specific to melanoma: breast-ovarian cancer BRCA1-2 related, Li-Fraumeni syndrome retinoblastoma, Cowden syndrome
-astrocitoma melanoma syndrome

In the context of hereditary melanomas must therefore distinguish melanomas which occur in patients suffering from complex Heredo-familiar neoplastic, wherein the melanoma is however not the main malignancy, but is one of the cancers that can occur in these patients, from the melanomas arisen in patients suffering from complex pathological conditions in which, however, there is high probability that a melanoma occurs, and, finally, from the forms of familial melanoma proper, in which the only neoplasm that really occurs in these subjects is the melanoma.

There are some hereditary cancer syndromes where family-specific mutations expose the patient to the onset of various solid tumors including melanoma. For example:

- The Breast Cancer Linkage Consortium showed an increased risk of melanoma in carriers of the BRCA2 mutation which predisposes mainly breast and ovarian cancers. (the Breast Cancer Linkage Consortium, 1999); as reported in the results of this work, also our data show that the BRCA mutation predisposes to melanoma.

- - In Li Fraumeni syndrome, where there is the mutation of the P53 gene (autosomal dominant) in addition to the onset of sarcomas, breast cancer, cerebral and adrenal, there has been an increased incidence of melanoma;
- - The same higher incidence of melanoma was found in patients with retinoblastoma,
- - Werner syndrome,
- - Cowden syndrome etc ..
- Other hereditary diseases in which it is highly likely the occurrence of melanoma are

- The dysplastic nevus syndrome familiar (FAMMM)

- Xeroderma pigmentosum and

- Albinism.

Familial melanoma

Clinical evidence that melanoma occurs in 10-12% of cases in family context (and that 3-5% of patients with a primary melanoma will develop a second melanoma in their lifetime) has led researchers, during the last twenty years, to talk about Familial melanoma and focus on cytogenetic and molecular biology studies that allow to identify some genetic alterations related to melanoma in familiar setting. Familiarity is an important risk factor; for melanoma, like other cancers, have first-degree relatives affected by tumor results in an increased risk of developing the same disease during their lifetime. (Sargent M.et al, 2014; Goldstein A., 2007)

The presumptive diagnosis of familiar melanoma, according the established criteria by the Italian Society of Human Genetics (SIGU-ONC, Section Guidelines, protocols http://www.sigu.net) and GenoMEL Consortium (Leachman SA. 2009), is made when the following data are detected from personal and family history:

a) two or more cases of melanoma in the same family.

b) It is already known a mutation in a gene of susceptibility to melanoma (eg. CDKN2A, CDK4);

c) Presence of multiple melanoma;

d) dysplastic nevus syndrome (or atypical mole) and melanoma (in the patient or in the family with DNS);

e) the presence of melanoma at an early age and a family member with cancer of the pancreas (some forms are related with the CDKN2A mutation).

In low incidence of melanoma populations (like South Europe) the diagnosis of familial melanoma can be made if there are at least two first-degree relatives or 3 second-degree relatives in the same branch of the family tree affected by melanoma (Brown et al , 2009), while in the regions with the highest incidence of melanoma, such as Australia, you can make the diagnosis of familial melanoma if there are at least three first-degree relatives in the same family with melanoma.

For the Italian population it has been estimated that individuals from families with multiple cases of melanoma have a risk of developing a melanoma about 50 and 25 times higher than the general population (whether it is respectively present or absent genetic alteration).

As in other malignancies, genes involved in the predisposition to melanoma, code for proteins that directly control the cell cycle and which, when mutated germline, significantly increases an individual's risk of developing melanoma.

As previously mentioned the etiopathogenesis of melanoma is multifactorial, then genetic factors play a role in synergy with environmental factors. It appears from clinical evidence that the mere presence of a germline mutation does not lead inexorably the occurrence of melanoma.

Genomic analysis of families with familial melanoma has clearly shown that not all members of the same family, carriers of germline mutations of genes at high risk, but residing in different geographic areas (or otherwise, exposed to "different external factors"), developed a melanoma; in these families, the carrier members, living in the same geographic area, developed melanoma, while the members of the family, always carrier of the same mutation, but emigrated to other nations, did not develop melanoma.

This confirms the hypothesis of the close synergy between genetic predisposition and environmental risk factors.

For the familial melanoma we can speak of "multifactorial inheritance with a threshold." The genetic predisposition and the environmental risk factors both help to determine the pathological phenotype and, likely, the severity of the disease. That is especially true for those mutations defined "on medium and low penetrance".

Some germline mutations in fact, more than others, confer susceptibility to the onset of melanoma; for the pathological phenotype be manifested it is necessary that one or more unfavorable genes together with one or more unfavorable environmental factors, added

together their pathological effect until reaching a threshold beyond which the disease occurs.

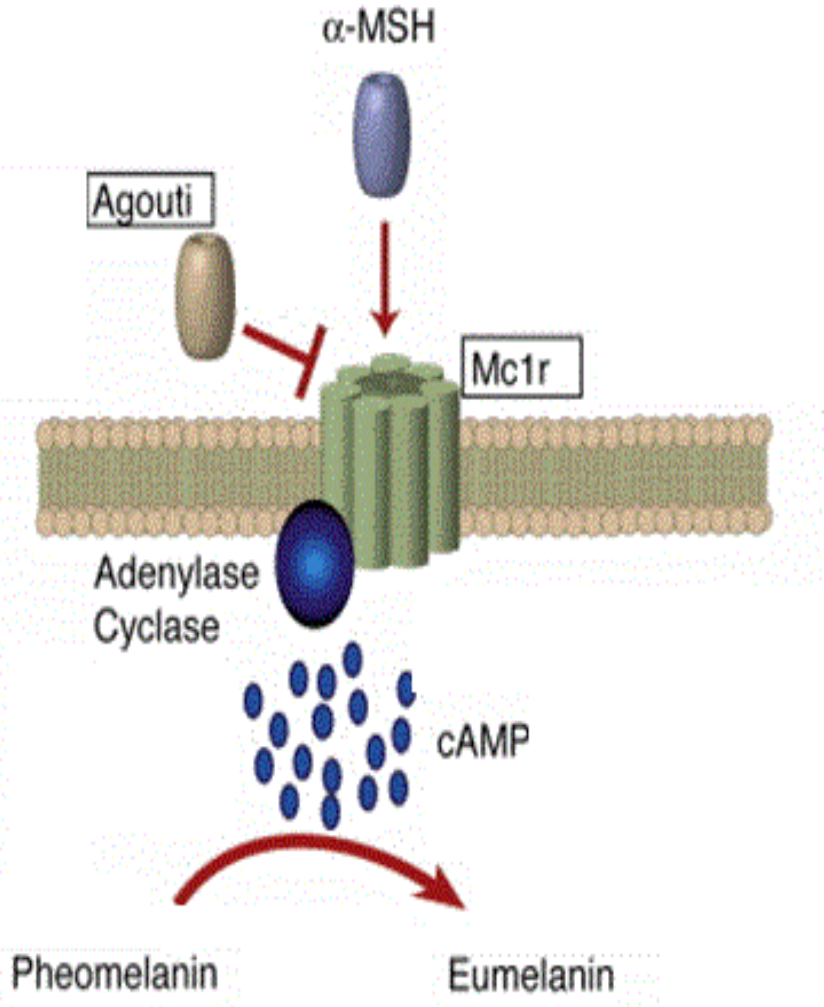
The cancer cells usually contain several mutations, but only a minority are driver mutations. The driver mutations are genetic mutations, essential for the genesis and development of the cancer. All other mutations, not fundamental to the development of cancer, are called passenger mutations. (Neil F et al, 2001)

To date, genes involved in the genetic predisposition to melanoma identified are divided into high-penetrance, medium penetrance and low penetrance genes, depending on their ability to confer a high risk, a medium risk or a low risk for the development of disease in individuals carrying the mutated genes.

High penetrance	CDKN2A; CDK4
Medium penetrance	MC1R; MTTF
Low penetrance	MTAP; EGF; GSTs; GST M1; GST T1; CYP2D6; VDR (vit.D receptor); TYR; ASIP; TYRP1; OCA2 (oculo-cutaneous-albinism) POt1;

The high-penetrance genes are the CDKN2A and CDK4 (which will be described in more detail later). they give to carrier a high risk of developing melanoma.

Among the medium penetrance genes the best known is the MC1R, or the gene coding for the melanocortin receptor.



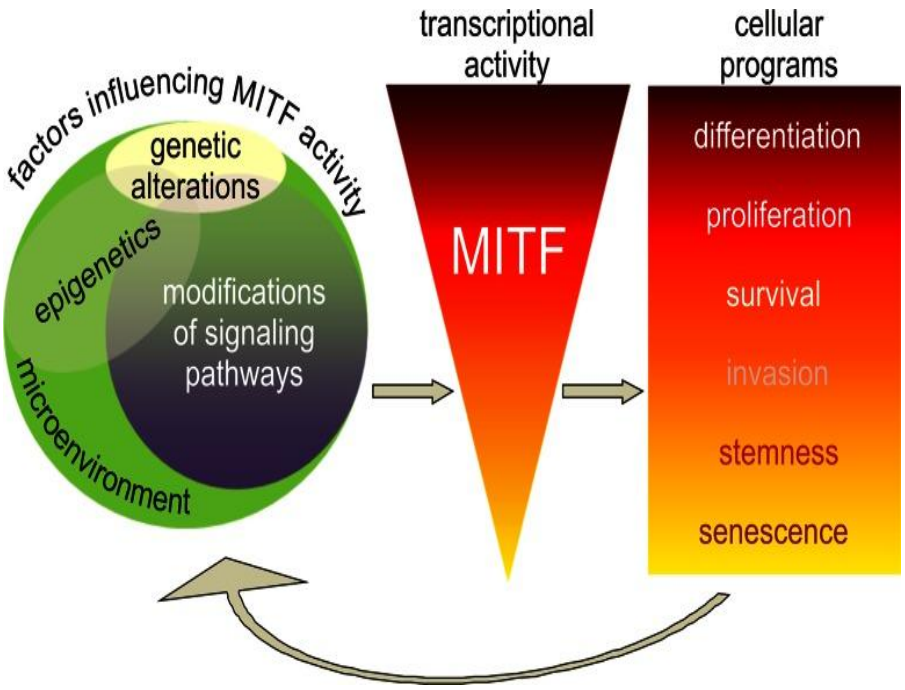
The MC1R gene is located on chromosome 16q24.3 and encodes the hormone receptor stimulating the melanocytes (MC1R); a receptor of 317 amino acids, which transduce the signal with a mechanism coupled to the protein G. The receptor is expressed in different cell types, including melanocytes and follicular keratinocytes and plays a key role in the regulation of skin pigmentation. The skin pigmentation is determined by the amount of melanin synthesized by epidermal melanocytes, and is recognized as a mechanism of skin protection against DNA damage induced by sun exposure. Melanin is synthesized by melanosomes that are inside the melanocytes. There are two forms of epidermal melanin, eumelanin (which has black - brown) and pheomelanin (red - yellow).

The link between MC1R and its ligand, hormone α-melanocyte stimulating (αMSH), activates adenylate cyclase and produces an increase in the level of cAMP. (Sturm, 2002).

The high concentration of cAMP leads to activation of protein kinase A, which in turn induces an increase in the transcription of the gene MITF (microphthalmia-associated transcription factor) involved in the transformations of pheomelanin in eumelanin. This gene is highly polymorphic gene and some of its variants, usually associated with Pale-red skin types and identified by the abbreviation RHC (Red Hair Color), result in a lower production of eumelanin (compared to feumelanina) after UV exposure. The melanocytes that express a variant of MC1R RHC type show greater sensitivity to cytotoxic effect of UV radiation. The combination of the presence of RHC variants and intermittent exposure to ultraviolet solar, is considered responsible for the activation of the oncogene BRAF, through an induced increase in intracellular levels of cyclic AMP.

These variants therefore, determining a minor photoprotection of the skin, are associated with a high risk of skin cancer. The variants of the MC1R confer risk of melanoma much lower than the CDKN2A, but are common in European populations. RHC variants are associated with an increased risk of developing melanoma in carrier individuals (OR 2.4) (Jhappan et al., 2003) (Demenais et al., 2010).

Recent studies have shown that the presence of a variant of MC1R in addition to a mutation CDKN2A significantly increases the risk of melanoma anticipating the onset of the disease at least 20 years, compared to individuals with a mutation CDKN2A alone. (Box et al., 2001). Less known than the previous gene, but today the focus of cancer research, is the MITF gene (transcription factor associated with Microphthalmia) coding for a regulator of differentiation, proliferation, migration and senescence of melanocytes. The MITF gene is located on chromosome 13 (Hartman et al., 2015; Garraway et al., 2005; Ugurel et al., 2007)



Genetic, epigenetic and the microenvironment alterations influence the expression of MITF and its activity in the melanoma cells, intervening consequently on the cellular differentiation (Bertolotto et al., 1998), proliferation (Carreira et al., 2005), and senescence.(Cheli et al., 2010).

MITF can control the expression of several genes involved in cell survival (HIF-1, BCL-2, MET, APE-1) (Beuret et al, 2007;. Busca et al., 2005;. Liu et al, 2009), cytoskeletal remodeling and cell migration (Carreira et al., 2006) and cell proliferation (CDK2) (Du et al., 2004). In addition, the MITF activity is related to resistance to apoptosis induced by UV in melanocytes

Only very recently the MITF gene was identified as a new susceptibility gene for melanoma. Recently, the sequencing of the entire genome in probands of families affected by melanoma, who did not have mutations in the genes CDKN2A and CDK4, allowed the identification of a new variant (E318K) of susceptibility gene for melanoma with intermediate penetrance. The gene encodes the microphthalmia-Associated Transcription Factor (MITF).

Two case-control studies, conducted within a large Australian and English population, have also confirmed the association between the E318K variant and the increased risk of developing melanoma in the general population. (Bertolotto et al, 2011; .. Yokoyama et al, 2011; Xu et al., 2000).). These results emphasize the role of the mechanisms involved in the regulation of pigmentation. The alterations of these mechanisms are involved in the predisposition to develop melanomas and in tumorigenesis through complex genetic-environmental interactions.

Low penetrance genes

Numerous studies today have revealed mutations of low penetrance genes, which by themselves are not able to induce the onset of melanomas. (Barrett et al., 2015). The heredity for this gene is multifactorial with a minimum threshold. External factors, or other minor gene mutations, contribute in determining the phenotype. Mutations of low penetrance genes are much more represented in the general population. Today, research is mainly done on mutations of genes involved in the regulation of pigmentation.

The ASIP gene, for example, encodes an antagonist protein of alpha-MSH.

The OCA gene encodes a membrane protein involved in the transport of tyrosine, melanin precursor.

Many researchers are now focusing on polymorphisms in the gene coding for the vitamin D receptor (VDR), which seems to play an important role in melanomagenesi. (Orlow et al. 2012).

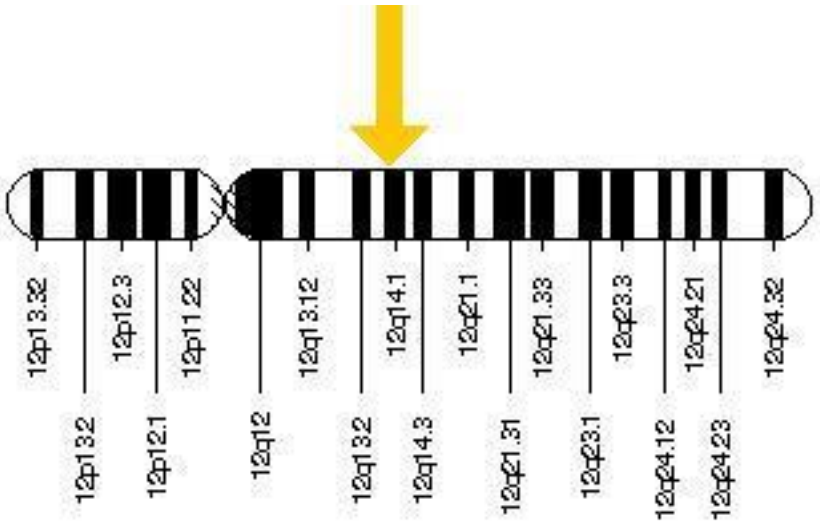
One of the genes most studied recently is the POT1. This gene (of which in reality little is known and we still do not know if it is a low-penetrance gene or not) encodes a nuclear protein involved in telomere protection, adjusting the length and integrity of telomeres. It protects chromosome ends from structural alterations that could produce catastrophic effects for the cell. Through next-generation sequencing, we identified three different mutations in the gene POT1 in a cohort of Italian patients with familial melanomas and two different mutations in French and American patients (Janxin et al, 2014).

Many other genes are now being studied and in the near future they may be able to explain the occurrence of melanomas in some family clusters. (KOSINIAK-Kamysz and Agnieszka et al 2014)

CDK4

The CDK4 gene is an oncogene, located on chromosome 12, in position 14, and encodes a cyclin-dependent kinase essential in the transition from the G1 to S phase of the cell cycle.

The gene consists of eight exons of which the first exon is a non-coding exon. The starting codon is at the beginning of exon 2 and the stop codon at the beginning of exon 8.



The most frequent mutations are in exon 2 codon 24, with a substitution of arginine with cysteine (Arg24Cys), or arginine with histidine (Arg24His).

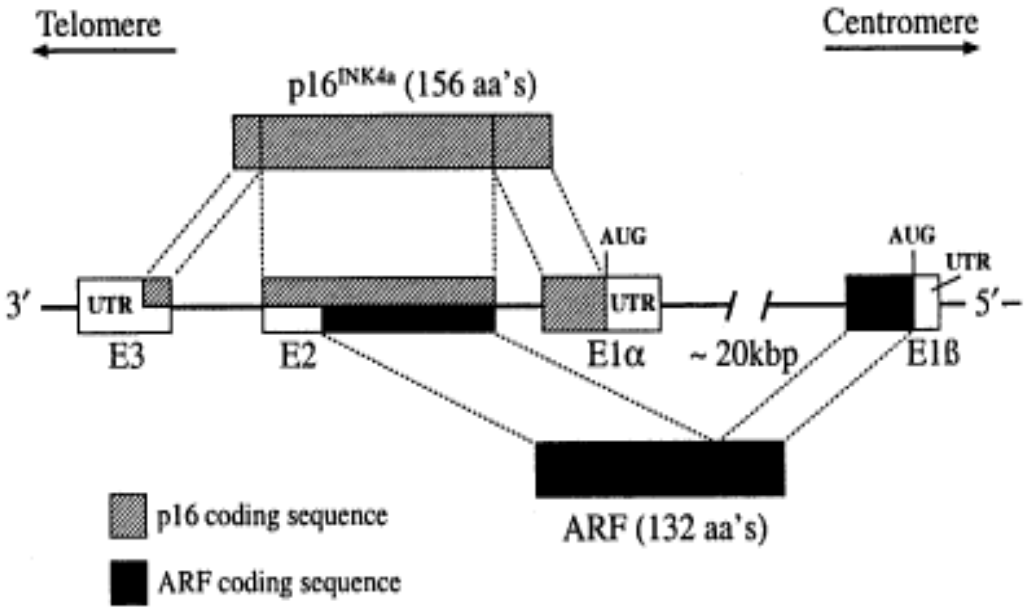
The very low frequency of mutations in the CDK4 gene in familial melanomas, suggests that this gene is a rare cause of hereditary melanomas and in some centers is not even screened. (Zuo et al., 1996, Rane et al., 2002)

The CDKN2A gene is instead the most common high-risk melanoma gene identified in malignant familial melanomas. According to national and international data, germline mutations in the coding region of the CDKN2A gene are identifiable in 20% - 40% of families with two or more members with melanomas, (Hashemi et al., 2000), a specific mutation has not yet been identified in the remaining 60-80%; in a minimum percentage instead (about 1%) there is a mutation of other known genes.

Therefore, in 20-40% of familial melanomas a mutation of the CDKN2A gene is present.

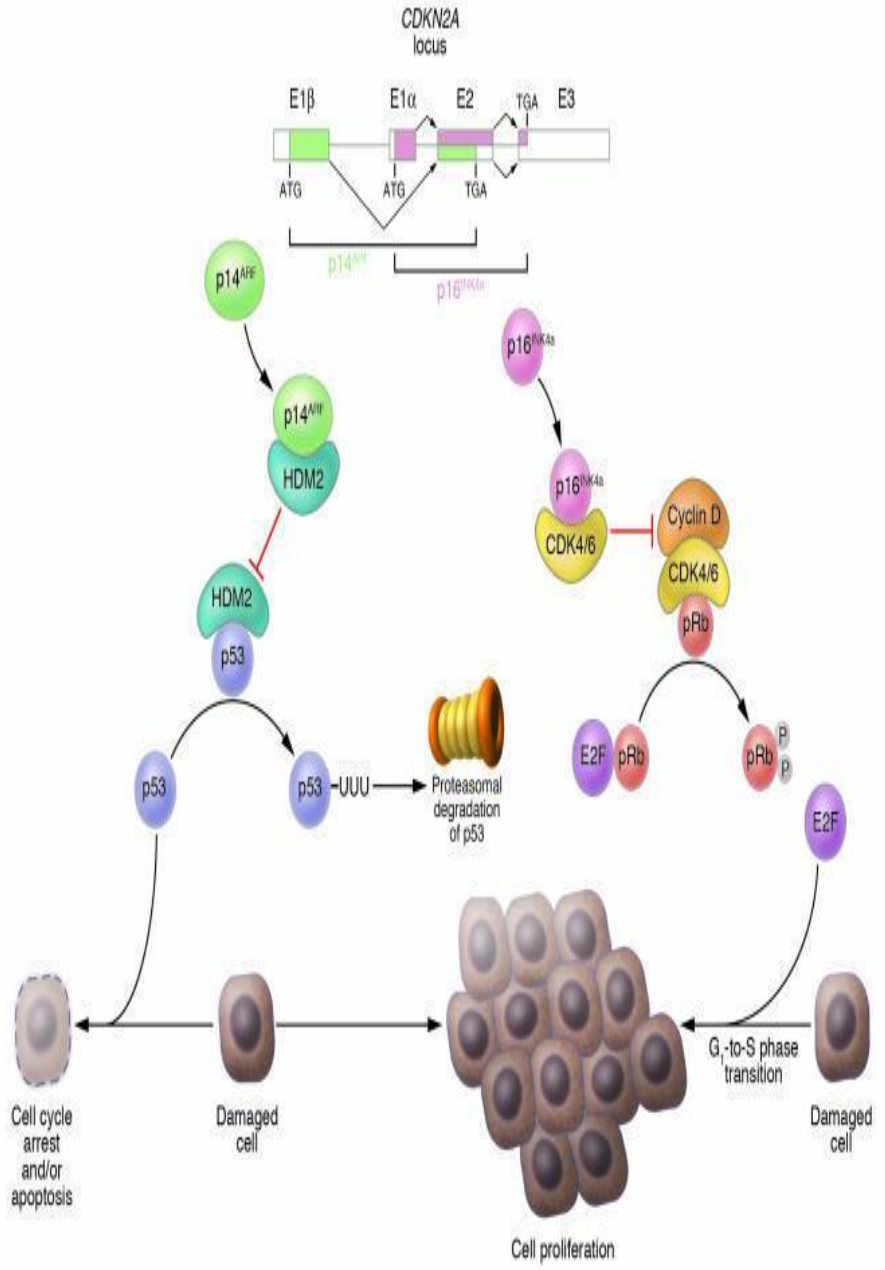
In 1994, in families with more members affected by melanomas, a mutation of CDKN2A, a tumor suppressor gene, was, for the first time, identified, localized in the 9p21 locus (the short arm of chromosome 9).

The CDKN2A gene encodes two distinct proteins, the protein-p16 INK4a (alpha transcript by exons E1α, 2 and 3) and p14 ARF (transcript from alternative splicing from exon 1beta (E1β, 2 and 3), which act as tumor suppressors, on two different pathways involved in cell cycle control.



P16 is encoded by the transcript of exons 1α, 2 and 3. P16 ties together the cyclin dependent kinases 4 and 6 (CDK) and thus inhibits the phosphorylation of the retinoblastoma protein, and blocks the passage of the cell from G1 to S phase cell phone.

Mutations in the CDKN2A gene can inactivate its inhibitory function and thus allow the cells to escape the block in G1 phase and promote uncontrolled cell growth.



The p14ARF (alternative reading frame), synthesized by alternative splicing of exon 1β with exons 2 and 3, determines the blockage of the passage from G1 to G2 phases of the cell cycle. It interacts with proteins Murine Double Minute 2 (MDM2) that binds p53 and prevents its degradation, leading to increased intracellular levels of P53 that, in response to genotoxic damage, induces cell apoptosis.

The deactivation of p14, as a result of mutations in the CDKN2A gene, leads to the accumulation of high levels of HDM2 and therefore to the loss of function of p53, with consequent activation of the processes of cell division. (Laud et al., 2006; Pavletich, 1999)

Mutations of the gene CDKN2A alter the main pathways of cell cycle regulation: the RB and P53 pathways. (Chin et al., 2006; Kamb et al., 1994)

Most of CDKN2A mutations are missense and occur in exons 2 and 1α, thus affecting mainly the p16 protein. (Begg et al., 2005; Hayward, 2003). In a small percentage of cases, with no coding mutations of CDKN2A, mutations have been identified also in some non-coding regions, as in the untranslated region at the 5 '(5' UTR), in the 3'UTR region and in the two introns that influence translation, initiation and splicing. (Balogh et al., 2012; Djursby et al., 2014; Harland et al., 2001; Veinalde et al., 2013).

Mutations in the exon 2 of CDKN2A affect the function of both proteins p16INK4A and p14ARF. The majority of mutations are missense and are identified in exon 1α and in exon 2;

numerous studies have shown a specific role in the predisposition to melanomas for the protein p14 ARF; in a small subset of familial melanoma, mutations were indeed found that affect only the p14 ARF (Garcia-Casado et al., 2009; Harland et al., 2005; Hewitt et al., 2002).

The frequency of mutations in the CDKN2A gene varies in different geographic areas: generally the rate is lower in areas with a sunny climate, and still ranges from 20% in Australia, 45% in North America to 57% in Europe.

Through segregation analysis an Autosomal Dominant (AD) pattern of transmission was found, with incomplete penetrance and variable expressivity. The CDKN2A gene is a tumor suppressor gene. This means that the inactivation of both alleles of the gene for the onset of melanoma is necessary.

In the case of familial melanomas, the first mutation is therefore a germline mutation, inherited from one parent, the second is a somatic mutation and, generally, is a deletion, leading to the so-called "loss of heterozygosity" (LOH).

This transmission mechanism explains why some members of the same family, even if they have a high risk of developing melanomas, do not really develop, perhaps because they live in different regions (and therefore less exposed to external risk factors, responsible for the loss of heterozygosity).

57 different germline CDKN2A genes in families with melanomas were identified.

65% were missense mutations,

16% deletions,

7% insertions / duplications,

10% nonsense mutations or splicing.

The majority of mutations in CDKN2A are "founder mutations" as the G101W, that originated, probably, from a common ancestor to several families.

The mutation of the gene has also been associated to the onset of pancreatic cancer (pancreatic cancer- melanoma syndrome, M-PC). (Lynch et al., 2002).

The CDKN2A gene, at the moment, is the most clinically important for the diagnosis of genetic predisposition to melanomas. The screening test for gene mutations can be made in high-risk individuals.

Lechman highlighted considerable variability in the rate of mutation in relation to ethnicity and geographic location. The frequency of the mutation seems to be lower in areas with a sunny climate and in geographical areas with high overall incidence of melanoma. Also the penetrance of the mutation (thus the real risk of developing melanomas in carriers of the mutation) seems to be different in relation to the geographical area; for example in 80 year-old-patients the penetrance was 58% in Europe, 76% in the US and 91% in Australia, proving that the penetrance varies with the incidence rates of melanomas among different populations.

As previously mentioned, it is estimated that the incidence of the mutation is 20% in Australia (low latitude region with a high overall incidence of melanomas), 45% in North America and 57% in European countries. These findings have led to the adoption of different selection criteria for genetic counseling of patients with suspected familial melanomas in different geographical areas. (Bishop et al. 2007).

In Italy genetic counseling for suspected familial melanoma can be done in the presence of a personal or family history that meets potentially one or more of the following criteria (in Italian society of human genetics, SIGU):

1) two or more cases of melanomas in the same branch of the family;

2) known mutation in a predisposing gene (CDKN2A, CDK4);

3) multiple melanomas;

4) dysplastic nevus syndrome (or atypical mole) and melanomas (in patients with DNS or in his familiars)

5) melanomas at a young age and a first-degree relative with cancer of the pancreas.

In countries with high overall incidence of melanomas, the criteria are more restrictive and require the presence of at least three subjects, first-degree relatives with melanomas. The risk of developing melanomas over a lifetime, in the Italian population is estimated to be 0.5%, compared with 2% for the US, 3.3% in Australia and 5.7% in New Zealand.

Due to the low overall incidence of melanomas in our region it is more likely that two first-degree relatives with melanomas have inherited a genetic mutation rather than having two cases of sporadic melanomas (hence the less restrictive criteria than Australia and New Zealand). The mutation frequency is related mainly to the number of family members with melanomas, ranging from 25% in families with 2 people with melanomas to 72% in those with 4 or more affected members. (Maubec E. et al. 2012)

The onset of melanomas at a young age, in the absence of a family history does not seem, however, to be a strong indicator of mutations in the CDKN2A gene, indeed several studies have shown a low prevalence of these mutations in patients with an early onset of melanomas. The association between pancreatic cancer and mutations in the CDKN2A has been proposed as a new heritable cancer syndrome ("Familial Atypical Multiple Mole Melanoma Pancreatic-Cancer), since these two malignancies are frequently observed simultaneously in patients. (Lynch 2002).

Why is it important to identify familial melanomas and carriers of high penetrance mutations?

International researches have shown that carriers of CDKN2A mutations from families with melanomas have a relative risk of developing melanomas in their lifetime (from 0 to 80 years) ranging from 58% (in Europe) up to 91%. While in sporadic melanomas the risk of melanomas increases with age, in familial melanoma, instead, the younger the patient is with melanoma in a family (early onset), the higher the relative risk becomes in melanomas developing in young relatives.

The identification, through a genetic test, of a familial predisposition to developing melanomas, could be very helpful in the primary prevention of melanomas (through removing environmental or esogen risk factors) and secondary prevention of melanomas (so called "early diagnosis") (Fallah et al. 2014).

The genetic study may also, in a near future, guide the development of therapeutic strategies (targeted therapy) with specific drugs that act electively on the molecular pathways altered by a specific genetic mutation. (by Hariharan, Vidhya, "Chemoprevention of Familial Melanoma" (2011). Dissertations (6 month embargo). Paper 1. http://ecommons.luc.edu/luc_diss_6mos).

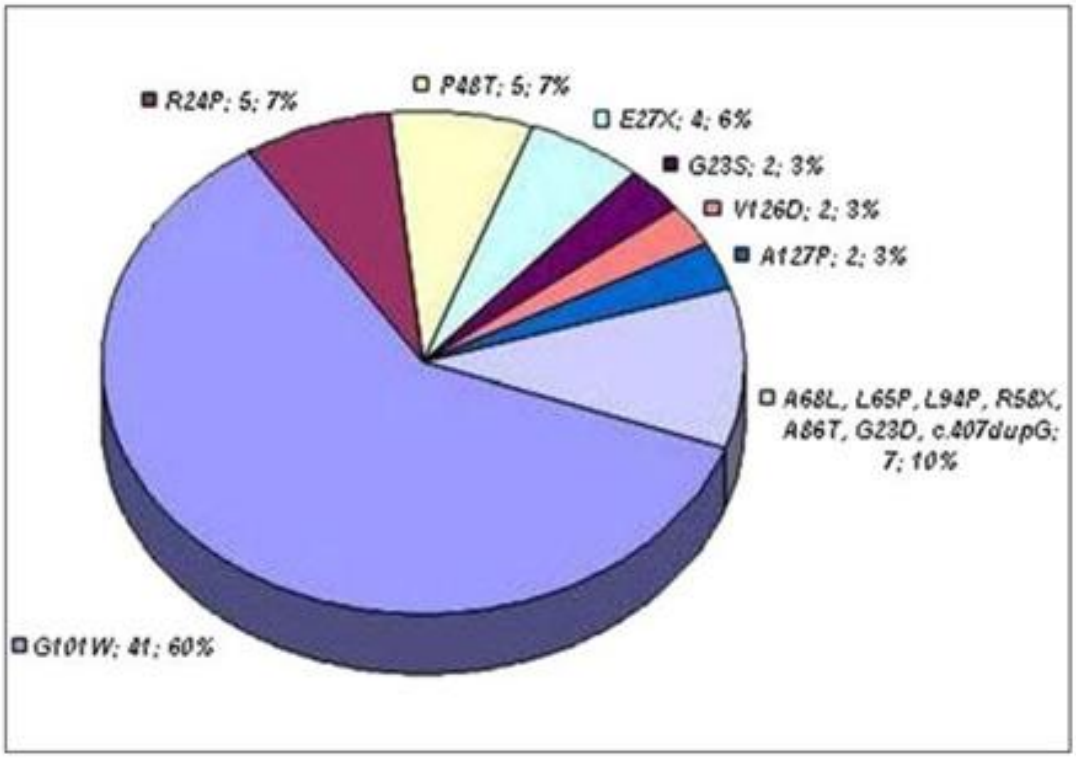
Since currently, the early diagnosis of melanomas is the best treatment options for the patient, the main goals of genetic screening and counseling are:

- Characterizing the genetic profile responsible for predisposition to melanomas,

- Identifying high risk subjects who can be included in dermato-oncologic follow-ups,

- Indicating correct behavior that does not expose the subject to additional risk factors that may increase the penetrance of the mutation (sunburns and tanning beds etc).

A recent study (Bruno et al.2009) summarizes the results of cytogenetic studies in 208 families with familial melanomas (which meet the SIGU criteria, illustrated above) of the following Italian regions: Piemonte, Liguria, Lombardy, Emilia Romagna, Lazio, Campania , Tuscany and Puglia; genetic screening highlighted that mutations in the CDKN2A gene are present in 33% of familial melanomas (68 families) and that at least 14 different types of mutations of this gene have been identified; of these, the G101W represents about 60%. This is a missense mutation, called "founder effect" which is also represented in French and American families. The haplotype analysis suggested that these genotypes are derived from a single ancestral haplotype in which the mutation first occurred (founder), and it is likely that this change has taken place in southwestern Europe. Other frequent mutations are: E27X, G23S, R24P P48T.



No family, as expected, shows alterations in the sequence of CDK4 gene.

Similar data on our region do not exist or are incomplete. Among the objectives of this project are: identifying and submitting to genetic screening all families with Familial Melanoma (MF) in our region; identifying the percentage of carriers of the mutation of the gene CDKN2A in Sicily; identifying the type of mutation present; assessing the role of other risk factors in determining the cancer.

It may be that the results of genetic testing in melanoma families in Sicily are not perfectly comparable to those of the rest of Italy and it is likely that, because of the different historical backgrounds, western Sicily presents different mutations to eastern Sicily.

A retrospective analysis of clinical data of 450 patients with melanomas treated at the Department of Surgical, Oncological and Oral Disciplines of the University of Palermo, in the period from January 2009 to August 2015, showed that fifty-six patients had a familial melanoma, or met the criteria of the Italian Society of Human Genetics for the clinical diagnosis of familial melanoma. Fifty-six selected patients were subjected to a genetic screening for the mutation of high penetrance genes, CDKN2A and CDK4.

From medical records, from interviews and histo-cyto-pathological reports, it was possible to:

- draw up a family tree

- identify other neoplastic diseases (personal or family)

- identify the external risk factors (or environmental) that have potentially induced loss of heterozygosity (LOH) and therefore favored the onset of melanoma within a family.

- evaluate the average age at diagnosis

- estimate the most common histological types

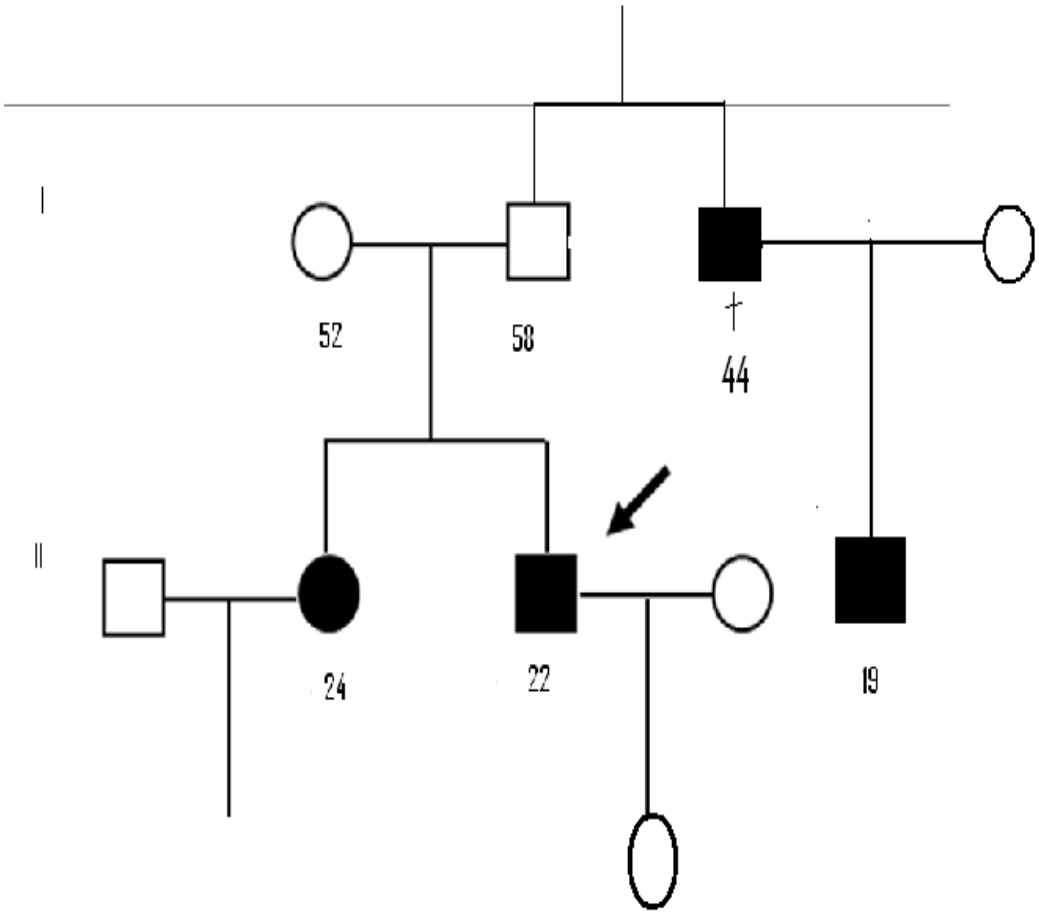
- evaluate the stage of the disease (and therefore the prognosis) at diagnosis.

A multidisciplinary team selected 56 patients with melanomas who had the criteria for the diagnosis of familial melanoma, and suggested starting specific genetic counseling to them, designed to assess the risk of genetic disease and the presence of a specific mutation of one of the two high-penetrance genes CDKN2A and CDK4.

Genetic counseling, performed with the oncogenetist, planned three meetings with the patient.

At the first meeting:

- a family tree was developed of the patient extending to three generations to second-degree relatives of the proband.



Example: 3 cousins, aged 24, 22 and 19, developed a melanoma, with a negative genetic test. Anamnesis showed that the father of one of the 3 patients, at age 44, had died of melanoma.

- Personal data, associated malignancies and the age of onset of any cancer (documented), and so the causes of death were noted.

- the patient's history is assessed with a focus on specific risk factors for melanoma, personal (presence of nevi or atypical moles) and environmental (occupational exposures or sunburn before puberty, use of tanning beds etc).

- the risk of disease is estimated. The proband's objectives were explained, the potential and limitations of genetic testing (informed consent to testing). For each patient, and this is

very important, it was explained that a negative test result (i.e. that shows no mutations in the genes screened), does not exclude the presence of other unscreened genetic mutations, if testing was instead positive, it would be possible to extend screening to family members.

At the second meeting, the patient signs the informed consent form to the test.

A venous blood sample is carried out. DNA is extracted from peripheral blood for genetic testing. The entire coding sequence of the CDKN2A (exons 1 alpha, 1 beta, 2 and 3), the intron-exon junctions, and the 5'UTR region are analyzed. In the CDK4 gene exon 2 is analyzed (in which the few recurrent mutations detected up till now are located). At the third meeting the test result is given. Whatever the result of the test, the proband is indicated the genetic risk related to the presence of a mutation (in the case of a positive test) and, in case of a negative test, however, the risk related to familiarity for melanoma.

Patients are indicated prevention strategies (eliminating behavioral risk factors, etc.) and indicated the diagnostic-preventive and therapeutic treatments. Psychological care is provided to all patients tested.

All patients accepted genetic screening; the results of 6 of the 56 tests are not yet ready, so only the results of 50 tests were evaluated in this work.

sequencing analysis

Germline mutation screening of the *CDKN2A* and *CDK4* genes was carried out. Genomic DNA was extracted from the whole peripheral blood of patients with familial melanomas using the QIAamp Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA yields and purity were determined spectrophotometrically by measuring the absorbance of aliquots at 260 and 280 nm. All DNA samples were of sufficient quality to be genotyped. Direct sequencing of the PCR products of four exons (1α, 1β, 2, 3) of *CDKN2A* gene and exon 2 of *CDK4* gene were performed using a BigDye Terminator v3.1 and then sequencing by ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Each genetic variant was confirmed by direct sequencing analysis on two independent peripheral blood samples. Data analysis was performed using the Sequencing Analysis 5.1.1 and Run 3100 Data Collection v2.0 softwares.

Results

Very interesting data came out from the analysis of the family trees, clinical data (age at diagnosis, skin phenotype, presence of other cancers etc), and the histological and genetic test results.

Patients		50
	Sex Male	25
	Female	25
Anatomical site	Head -neck	2
	Trunk	30
	Limb	18
Istotype	Mel in situ	11
	SSM	26
	Mel Nodular	13
One Melanoma		40
multiple Melanomi		10
	Sincronous	3
	Metacronous	7 di cui
		6 Mel in situ
		5 Mel < 1mm
		1 Mel >1 mm
Breslow thickness of primary melanoma		
	Melanoma in situ	11

	Mel < 1mm	20
	Mel 1 - 2 mm	9
	Mel > 2mm	10
AJCC Stage	**0**	11
	Ia	14
	Ib	14
	IIa	3
	IIb	4
	III	3
	IV	1
Age of onset		
	<20	5
	20-30	8
	30-40	20
	40-50	10
	>50	7
Familiarity		
	- **< 2**	45
	- **>2**	5
Other cancer		4
	Mieloide Chronic Leukemia mieloide	1
	Breast Cancer	3 (BRCA 2 pos)
	Screening of CDKN2A mutation	**1**
	Screening of CDK4 mutationn	0

Some results are similar to those reported in literature, some are not. The incidence of familial melanomas in our series was 11.11%, which is coherent with the percentage reported in the literature (10-12%).

All patients had a pale skin type, type I-III of Fitzpatrik, and 4 patients had red hair.

Almost all patients had a history of unprotected sun exposure (beach) or even sunburn in adolescence. Work does not seem a risk factor (few insignificant cases of rural workers or fishermen).

No cases of occupational exposure and environmental risk factors. In our series the familial melanoma affects men and women equally. The mean age at diagnosis was 35.8 years (range 15-60 yr).

In 60% of cases the melanoma occurs on the trunk, in 36% of cases in the limbs and 4% in the head and neck. 52% of melanomas are histologically, a Superficial spreading melanoma, while 26% are nodular melanomas and 22% melanomas in situ. No cases of lentigo malignant melanomas or Acral melanomas were observed among patients who met the criteria for genetic counseling. Also these data are widely consistent with those reported in literature (M. Sargent et al, 2014).

Ten of the patients had multiple lesions; in 3 patients there were more melanomas simultaneously at the time of diagnosis (Synchronous melanomas) and in 7 cases a second (and a third in one case) primary lesion appeared during the follow up period (melanoma metachronous). Almost all multiple melanomas were histological melanomas in situ or thin melanomas diagnosed during the follow-up.

The stage of the disease in patients with familial melanoma is very interesting. The majority of our patients, in fact (62%), had a thin melanoma at the time of diagnosis (22% melanoma in situ, 40% melanoma with Breslow thickness <1mm); 18% had a melanoma with Breslow thickness between 1 and 2 mm; 20% a melanoma with Breslow thickness > 2mm.

18 melanomas (36%) had histological ulceration. Four patients died (8%).

Most patients, 78%, at diagnosis were in stadium O - I according to the AJCC staging system, 14% stage II, while only 4 patients (8%) were in advanced stages of the disease (III-IV) .

The interpretation of this finding is not simple.

Why is the familial melanoma frequently a thin melanoma?

Is it possible to assume that the familial melanoma has a different biological behavior, more benevolent than the sporadic melanoma and thus, obviously, a more favorable prognosis?

Is the thin thickness of melanomas at diagnosis the result of an early diagnosis? Or due to the greater attention on pigmented lesions of family members of patients with melanomas (self-examination, dermatologic visits)? To date we are not able to answer these questions.

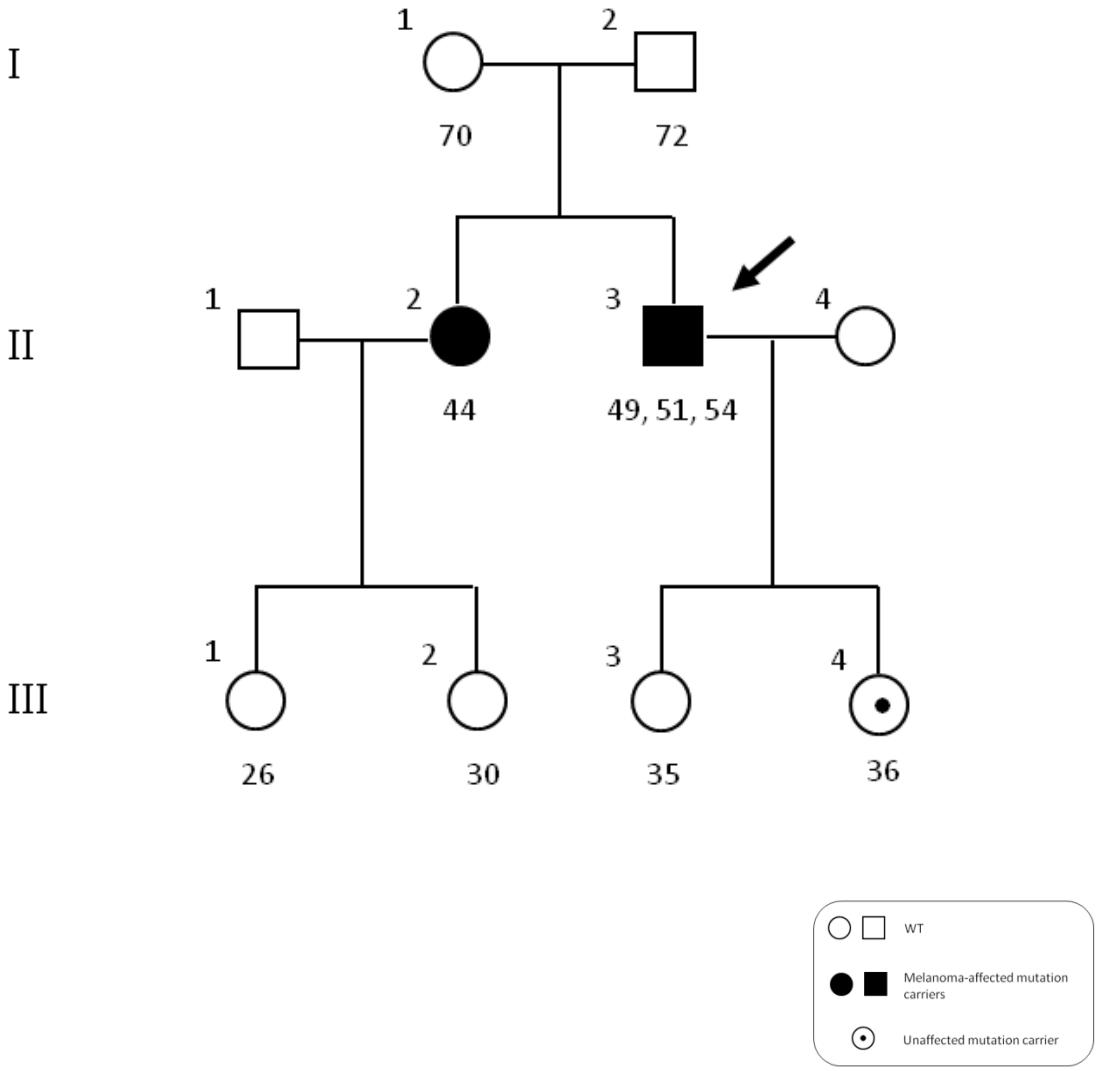
A significant medical history was highlighted in only three cases. Three women with familial melanoma, negative for germline mutation of CDKN2A and CDK4 genes, had a personal history of breast cancer, BRCA1 Related.

Some recent reports in literature, in fact, associate the BRCA mutation with the onset of other non-mammary tumors including melanomas. Specifically, a higher incidence of melanomas are reported in literature in breast cancer survival carriers of mutations of BRCA1-2 genes with respect to the general population. This aspect definitely deserves further study.

Another important finding, consistent with the literature, is that 70% of patients have a high number of nevi (one or more dysplastic nevi removed).

But, without doubt, the least expected result is that related to the genetic test: only one of the fifty patients screened was a carrier of the mutation in the high penetrance gene CDKN2A. No patient, as it was expected, was a carrier of the mutation CDK4.

If the incidence of CDKN2A mutation in familial melanomas of our region was, as reported in national and international literature, 30%, the expected result would have been different. At least 15 patients should be carriers of the mutation, and not only one.



The proband, positive for the mutation, has a primary melanoma, pT2aN0M0, and 3 more primary melanomas, metachronous, thin and in situ. A positive test of the proband permitted extended genetic screening to family members of the proband and the same mutation was identified in the daughter (healthy carrier) and the sister carrier of the mutation but affected by melanoma.

The mutation R87w was found, in exon 2 of the gene CDKN2A. It is not one of the most frequently detected in Italy (G101W, 60%, R24P, 7%, P48T, 7%, E27X, 6%) as published by Bruno et al in 2009. The R87W mutation is unusual. This type of mutation, which causes the substitution of arginine to tryptophan at position 87, has not yet been formally associated with familial melanomas and in literature has been described in only two cases in Spain and Greece.

Melanomas are the most serious and aggressive form of skin cancer whose incidence is increasing in recent decades. The prognosis of patients with cutaneous melanomas is related to the stage of the disease at diagnosis. (Tsao et al., 2012); Melanomas in an advanced stage do not respond to conventional therapies (Bishop et al., 2007) for such prognoses they are often ominous. Primary and secondary prevention (early diagnosis) are the only effective strategies in the fight against melanomas (Bhatia et al., 2014).

The identification of people at risk of developing a potentially fatal disease is extremely important.

In genetically susceptible people the reduction of behavioral and external risk factors, that can increase the penetrance of germline mutations, could lead to a reduction in the incidence of the disease; a careful oncodermatologic follow-up could allow an early diagnosis of the disease (at a curable stage).

Germline mutations of the CDKN2A gene, involved in cell cycle regulation, confer a high risk of malignant melanomas in carriers of the mutation (Della Torre et al., 2001). In addition to the high-penetrance genes, which confer a high risk of developing melanomas, other genes are also involved in the risk of developing melanomas.

Mutations and allelic variants of minor genes (MC1R, MITF, Asip etc) have in fact been identified and defined as susceptibility genes with a low to medium risk of developing melanomas, or as modifier-amplifier of high risk-susceptibility genes at (Udayakumar et al., 2010).

In most of the case series reported in the literature the high number of cases of melanomas in the same family, the early onset of melanomas (young age at onset) and the presence of multiple melanomas in the same patient (synchronous or metachronous) are strongly related to the presence (and to the inheritance) of the mutation of the CDKN2A gene, which is present in 20-40% of patients with familial melanoma (Tsao et al., 2000). The penetrance of the mutation, or the manifestation of the neoplastic phenotype in carrier

families of the mutation is 30% within 50 years, 67% at 80 years of age, but it seems highly variable in relation to the geographical area ; the risk appears higher among residents of the sunniest areas (Bishop et al., 2002; Fallah et al, 2014).

The analysis of our results indicates that:

- in our sample of Familial Melanoma (only Sicilian patients) the mutation of the CDKN2A gene has a much lower incidence than reported in literature
- in most of the familial forms thin melanomas are found in the early stage disease. The interpretation of this data is far from simple and raises many questions, which, based on our current knowledge, unfortunately, I suspect are not possible to answer.

Is it correct to assume a correlation between the thin Breslow thickness of familial melanomas and the absence of mutations of high penetrance gene?

Could we speculate that the thin thickness of melanomas may be related to mutations of minor genes (not screened and unknown) responsible for a less aggressive biological behavior of melanomas? As previously mentioned, a negative genetic test clearly does not exclude the hereditary nature of melanomas.

Can we assume that the familial melanoma, in our region, can be attributed to genetic mutations (not screened) of medium or low penetrance genes, which, along with environmental risk factors, induce the occurrence of thin melanomas? And, therefore, these melanomas have a potentially more favorable biological behavior and prognosis?

This hypothesis is also supported by the evidence that only 4 patients with familial melanomas, negative for mutations of high penetrance gene, died within 5 years of follow-up (patient with melanomas > 1mm, with ulceration at diagnosis).

As mentioned earlier, melanomas are a complex and heterogeneous disease and its pathogenesis is due to many genetic, individual and environmental factors.

Cytogenetic studies have highlighted the strong molecular heterogeneity of melanomas, identifying several gene mutations in different pathways, involved to varying degrees in melanomagenesis and, probably, responsible for the more or less aggressive biological behavior of the tumor. According to the hypothesis of a second model of

melanomagenesis ("non linear" model), some specific mutations in stem cells would directly cause the onset of melanomas to start growing vertically or already be metastatic, without passing through the stage of melanoma in situ or horizontal growth Melanoma(SSM); by the same principle a specific mutation could induce only the occurrence of thin melanomas or melanomas in situ.

But which genes to test?

Another question is: why is the incidence of the mutation in our regional sample so low?

In literature, it is estimated that the rate of mutation of the CDKN2A gene in familial melanomas of geographical areas with low overall incidence of melanomas, such as Europe and North America, ranges between 30% and 57%. Therefore, according to this data, in theory, the expected result in our sample would have been different: at least 14-15 patients should have had the genetic mutation (estimating minimum incidence, 30%) and that half of these should have had the mutation called G101W . (Goldstein et al., 2007, Capes et al., 2002; Puig et al., 2005; Ciotti et al., 2000)

Only one patient, in our sample, had a genetic mutation of the CDKN2A gene, but not the G101W as reported in most published series, but the mutation called R87w, a rare missense mutation in exon 2, which resulted in a substitution of arginine to tryptophan at position 87. To date the R87W mutation is not formally associated with the onset of familial melanomas.

Unlike the populations of other Italian and European regions, in the Sicilian families examined a very low frequency of CDKN2A mutation was found. This difference can be attributed to several factors such as, for example, the historical background of Sicily.

For its crucial geographic position, in the center of the Mediterranean, Sicily has been inhabited by different peoples, cultures and civilizations. Studies of allelic variants of hemoglobin in beta thalassemia (Giambona et al., 2011) have demonstrated the extreme genetic heterogeneity of the Sicilian people, probably a result of various dominations (Greeks, Phoenicians, Etruscans, Romans, Byzantines, Arabs, Normans, Aragon, Bourbons) that have occurred in the history of our region.

A recent Italian study (Casula et al 2007-2009), comparing the results of genetic screening of the CDKN2A gene in familial melanomas of other Mediterranean regions, Sardinia (Sassari) and Campania (Naples), revealed interesting data that seem to support our hypothesis; it seems that in Campania the frequency of the mutation tends to be lower than in Northern Italy, with a 17% mutation rate, while in familial melanomas in Sardinia the frequency of the mutation is virtually zero.

Such low mutational rates have been described only in the geographical areas with a high overall incidence of melanomas (such as Australia), in which the probability of finding the mutation of the CDKN2A in a familial melanoma is quite low and is estimated at 20%.

Probably, in geographic areas with a high overall incidence of melanomas, it is more likely that the Familial Melanoma is due to an inheritance of medium or low penetrance genes (as MTIF, MC1R) and to the exposure to the same environmental risk factors (sun exposure etc) of members of the same family, rather than the inheritance of high-penetrance mutations of genes. In Sicily there is not a high overall incidence of melanomas; in Italy, in fact, there is a decreasing trend in incidence, from the north to the south, with incidence rates 4-fold lower in the south than the north.

The latitude and greater exposure to the sun in our region (compared to other regions of Italy or Europe) probably represent environmental factors, which cause the loss of heterozygosity (LOH) of medium or low susceptibility genes and promote the onset of melanomas.

Our latitude and our history could make us genetically different to the European population.

Our findings have led us to hypothesize that a very low frequency of germline CDKN2A mutations could be a population-specific genetic signature for Sicilian patients affected by familial melanomas.

A larger number of patients enrolled for genetic testing and the introduction of new genetic screenings to identify other mutations in other moderate/low-penetrance susceptibility genes involved in the genesis of familial melanomas will aid in clarifying which are the most common gene mutations and the role of environmental factors in

determining this disease, and, not least, the prognostic significance of these mutations (thin melanomas vs thick melanomas).

The fight against melanomas is today entrusted essentially to early detection.

The identification of patients at risk and the implementation of primary prevention strategies are the only weapons available to cure melanomas.

Soon, research will further clarify the molecular mechanisms involved in melanomagenesis and biological mechanisms that regulate the development and progression of cancer. It will be possible to classify each melanoma, from the molecular point of view, and to give the patient the target therapy specific to the occurring molecular-genetic aberration.

Although the future of genetic research on melanomas is not too far away, and it seems that some objectives will be achieved soon, today, 190 years after Thomas Fawdington (1826) we ... "are hence forced to confess the incompetency of our knowledge of the disease under consideration, and to leave to future investigators the merit of revealing the laws which groom its origin and progress ... .and pointing out the means by which its ravages may be prevented or repressed. "

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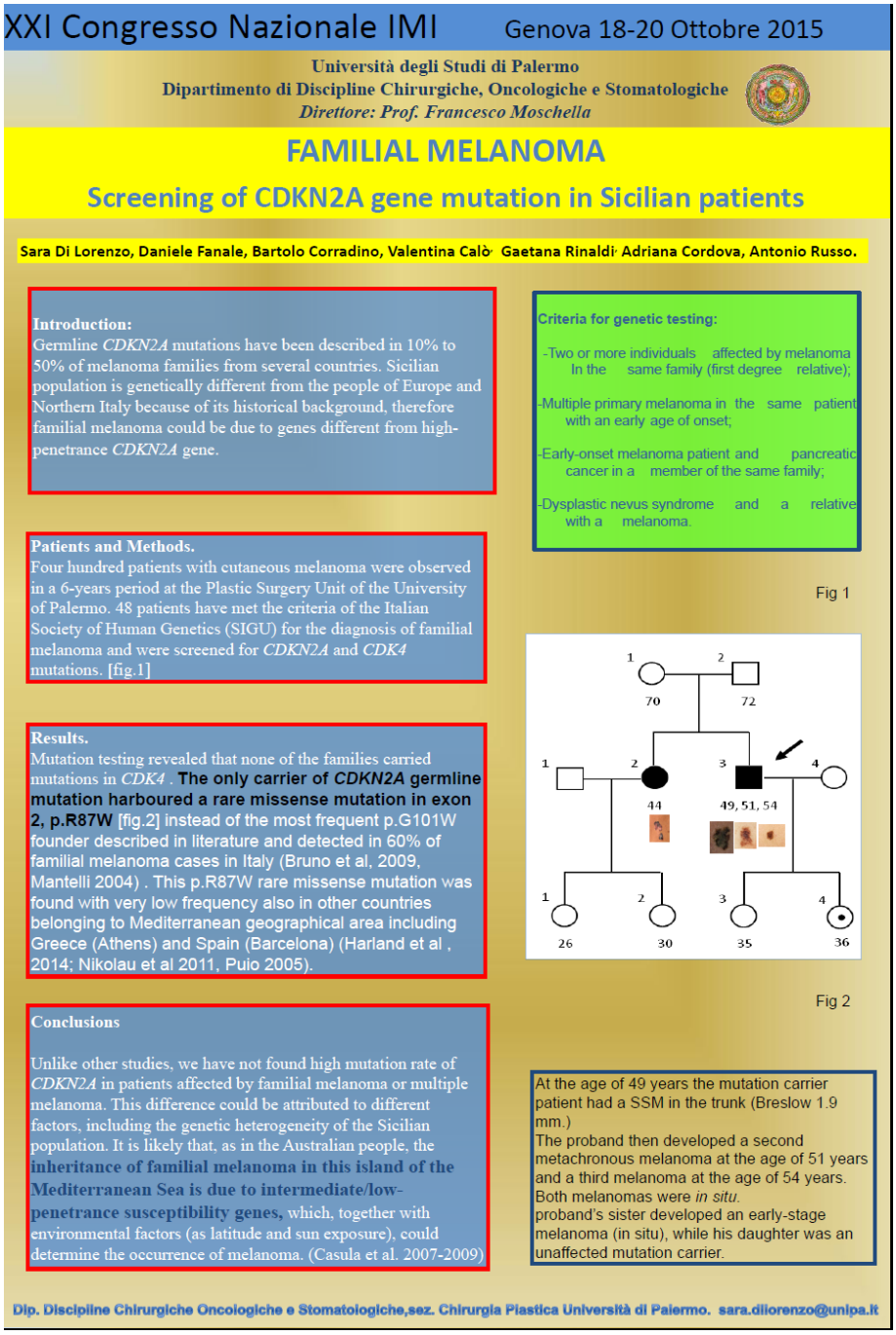
The results were presented:

- The National Congress of the Italian Society of Plastic Reconstructive and Aesthetic Surgery (SICPRE), skin cancer session. Milan in September 2015

- The National Congress of Italian Melanoma Intergroup (IMI), Genoa, October 2015 as posters,

The study entitled: "Absence of germline CDKN2A mutation in Sicilian Patients with Familial Malignant Melanoma: could it be a population-specific genetic signature? (Di Lorenzo S. et al) was published in the journal Cancer cell Biology and Therapy, December 2015 (attached)



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Università degli Studi di Palermo
Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche
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FAMILIAL MELANOMA

Screening of CDKN2A gene mutation in Sicilian patients

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Introduction:
Germline *CDKN2A* mutations have been described in 10% to 50% of melanoma families from several countries. Sicilian population is genetically different from the people of Europe and Northern Italy because of its historical background, therefore familial melanoma could be due to genes different from high-penetrance *CDKN2A* gene.

Criteria for genetic testing:

-Two or more individuals affected by melanoma In the same family (first degree relative);

-Multiple primary melanoma in the same patient with an early age of onset;

-Early-onset melanoma patient and pancreatic cancer in a member of the same family;

-Dysplastic nevus syndrome and a relative with a melanoma.

Fig 1

Patients and Methods.
Four hundred patients with cutaneous melanoma were observed in a 6-years period at the Plastic Surgery Unit of the University of Palermo. 48 patients have met the criteria of the Italian Society of Human Genetics (SIGU) for the diagnosis of familial melanoma and were screened for *CDKN2A* and *CDK4* mutations. [fig.1]

Fig 2

Results.
Mutation testing revealed that none of the families carried mutations in *CDK4* . **The only carrier of *CDKN2A* germline mutation harboured a rare missense mutation in exon 2, p.R87W** [fig.2] instead of the most frequent p.G101W founder described in literature and detected in 60% of familial melanoma cases in Italy (Bruno et al, 2009, Mantelli 2004) . This p.R87W rare missense mutation was found with very low frequency also in other countries belonging to Mediterranean geographical area including Greece (Athens) and Spain (Barcelona) (Harland et al , 2014; Nikolau et al 2011, Puio 2005).

Conclusions

Unlike other studies, we have not found high mutation rate of *CDKN2A* in patients affected by familial melanoma or multiple melanoma. This difference could be attributed to different factors, including the genetic heterogeneity of the Sicilian population. It is likely that, as in the Australian people, the **inheritance of familial melanoma in this island of the Mediterranean Sea is due to intermediate/low-penetrance susceptibility genes**, which, together with environmental factors (as latitude and sun exposure), could determine the occurrence of melanoma. (Casula et al. 2007-2009)

At the age of 49 years the mutation carrier patient had a SSM in the trunk (Breslow 1.9 mm.)
The proband then developed a second metachronous melanoma at the age of 51 years and a third melanoma at the age of 54 years. Both melanomas were *in situ*.
proband's sister developed an early-stage melanoma (in situ), while his daughter was an unaffected mutation carrier.

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