



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Clinical genetic testing for familial melanoma in Italy: a cooperative study

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Clinical genetic testing for familial melanoma in Italy: a cooperative study / W.Bruno, P.Ghiorzo, L.Battistuzzi, P.A.Ascierto, M.Barile, S.Gargiulo, F.Gensini, S.Gliori, M.Guida, M.Lombardo, S.Manoukian, C.Menin, S.Nasti, P.Origone, B.Pasini, L. Pastorino, B.Peissel, M.A.Pizzichetta, P.Queirolo, M.Rodolfo, A.Romanini, M.C.Scaini, A.Testori, M.G.Tibiletti, D.Turchetti, S.A.Leachman, G.Bianchi Scarrà; IMI, Italian Melanoma Intergroup. - In: JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY. - ISSN 0190-9622. -

Availability:

This version is available at: 2158/395264 since:

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

Clinical genetic testing for familial melanoma in Italy: A cooperative study

William Bruno, MD, PhD,^{a*} Paola Ghiorzo, PhD,^{a*} Linda Battistuzzi, MS,^{a*} Paolo A. Ascierto, MD,^b Monica Barile, MD,^c Sara Gargiulo, PhD,^a Francesca Gensini, MD,^d Sara Gliori, MD,^a Michele Guida, MD,^c Maurizio Lombardo, MD,^f Siranoush Manoukian, MD,^g Chiara Menin, PhD,^h Sabina Nasti, PhD,^a Paola Origone, PhD,^a Barbara Pasini, MD,ⁱ Lorenza Pastorino, PhD,^a Bernard Peissel, MD,^g Maria Antonietta Pizzichetta, MD,^j Paola Queirolo, MD,^k Monica Rodolfo, PhD,^g Antonella Romanini, MD,^l Maria Chiara Scaini, PhD,^m Alessandro Testori, MD,ⁿ Maria Grazia Tibiletti, MD,^o Daniela Turchetti, MD,^p Sancy A. Leachman, MD,^q and Giovanna Bianchi Scarrà, PhD,^a on behalf of IMI, the Italian Melanoma Intergroup
Genoa, Naples, Milan, Florence, Bari, Varese, Padua, Turin, Aviano, Pisa, and Bologna, Italy; and Salt Lake City, Utah

Background: The Italian Society of Human Genetics' (SIGU) recommendations on genetic counseling and testing for hereditary melanoma state that clinical genetic testing can be offered to Italian melanoma families with at least two affected members.

Objective: In the framework of a cooperative study, we sought to establish the frequency of *cyclin-dependent kinase inhibitor 2A* mutations in melanoma families that underwent clinical genetic counseling and testing in accordance with the SIGU recommendations at 9 centers in different Italian regions.

Methods: *Cyclin-dependent kinase inhibitor 2A* testing was conducted by direct sequencing and multiplex ligation-dependent probe amplification analysis in melanoma families with at least two affected members.

Results: A total of 33% (68/204) of the families harbored *cyclin-dependent kinase inhibitor 2A* mutations. In the 145 families with two affected members the mutation frequency was 25%. Three novel mutations, L94P, A86T, and c.407dupG, were identified among the cases and not in 200 controls.

Limitations: We were unable to perform separate analyses for individual centers, as in some cases the number of families was too small.

Conclusions: The availability of clinical genetic testing for melanoma to families with just two affected members in the same branch is justified in Italy in terms of the likelihood of identifying a mutation. (J Am Acad Dermatol 2009;61:775-82.)

Key words: *cyclin-dependent kinase 4*; *cyclin-dependent kinase inhibitor 2A*; familial melanoma; genetic testing.

From the Department of Oncology, Biology, and Genetics, University of Genoa^a; Medical Oncology and Innovative Therapy Unit, National Tumor Institute "Fondazione Pascale," Naples^b; Division of Cancer Prevention and Genetics, Chemoprevention, European Institute of Oncology, Milan^c; Section of Medical Genetics, Department of Pathophysiology, University of Florence^d; Medical Oncology Department, National Cancer Institute, Bari^e; Dermatology, "Ospedale di Circolo," Varese^f; Medical Genetics Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Nazionale dei Tumori, Milan^g; Molecular Immunology and Diagnostic Oncology, Istituto Oncologico Veneto, IRCCS, Padua^h; Department of Genetics, Biology and Biochemistry, University of Turinⁱ; Division of Medical Oncology, C-Preventive Oncology, Centro di Riferimento Oncologico, National Cancer Institute, Aviano^j; Department of Medical Oncology A, National Institute for Cancer Research, Genoa^k; Department of Oncology, Division of Medical Oncology, S. Chiara Hospital, Pisa^l; Section of Oncology, Department of Oncology and Surgical Sciences, University of Padua^m;

Melanoma and Muscle-Cutaneous Sarcomas Division, European Institute of Oncology, Milanⁿ; Anatomic Pathology Unit, University of Insubria, "Ospedale di Circolo," Varese^o; Medical Genetics, Bologna University, "Policlinico S. Orsola Malpighi"^p; and Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah.^q

*These authors contributed equally to this study.

Supported by the International Melanoma Genetics Consortium 01872 Network of Excellence Grant to Dr Bianchi Scarrà; Dr Scaini is supported by a Fondazione Italiana per la Ricerca sul Cancro (FIRC) fellowship.

Conflicts of interest: None declared.

Accepted for publication March 20, 2009.

Reprints not available from the authors.

Correspondence to: William Bruno, MD, PhD. E-mail: william.bruno@unige.it.

Available online June 5, 2009.

0190-9622/\$36.00

© 2009 by the American Academy of Dermatology, Inc.

doi:10.1016/j.jaad.2009.03.039

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is the major high penetrance susceptibility gene identified to date in melanoma families worldwide. Mutations in the other known high-risk melanoma susceptibility gene, *cyclin-dependent kinase 4 (CDK4)*, are very rare.¹

CDKN2A codes for two separate tumor suppressor proteins, p16INK4a and p14ARF, arising from alternative first exons (1 α and 1 β), that are spliced onto the common exons 2 and 3, but in different reading frames. Both proteins act as tumor suppressors, p16INK4a through the retinoblastoma cell cycle control pathway, and p14ARF through the p53 pathway. Most *CDKN2A* mutations are missense mutations located in the coding sequences of exons 1 α and 2, and many seem to derive from ancestral founders.²⁻¹¹

CDKN2A mutations have been found in 20% to 40% of melanoma families with 3 or more affected members.¹ However, the proportion of families with mutations varies between countries, depending on factors such as baseline melanoma incidence rates and family and population selection in studies. A recent collaborative study by 17 research groups belonging to the International Melanoma Genetics Consortium (GenoMEL) studied *CDKN2A* mutations across 385 families with 3 or more confirmed affected members,¹⁰ at least two of whom underwent mutational testing. Overall, 39% of the families were *CDKN2A* mutation-positive, ranging from 20% in Australia, to 45% in North America, and 57% in Europe. A high number of patients with melanoma per family, early age of onset, and the presence of multiple primary tumors showed significant associations with *CDKN2A* mutations, but the effects varied widely across continents.

In Italy, studies on smaller samples of families^{9,11-15} reported that *CDKN2A* mutations are found in Italian families with just two cases and in families with larger numbers of affected members.

Based on this background, we aimed to establish the frequency of *CDKN2A* and *CDK4* mutations in melanoma families that underwent clinical genetic counseling and testing at centers in different Italian regions in accordance with the Italian Society of Human Genetics' (SIGU) recommendations^{16,17} and,

therefore, to verify to what extent compliance with those recommendations results in testing of individuals with a reasonable chance of carrying a mutation. The second aim of this study was to evaluate the relationship between presence of *CDKN2A* or *CDK4* mutations, number of affected members in the family, age at first melanoma diagnosis (AAD), and presence

of multiple primary melanomas (MPM) in Italian probands and their families, to possibly contribute to an update of the SIGU criterion for candidacy to testing.

We thus report here the results of the first cooperative study on Italian melanoma families that participated in clinical genetic counseling and testing.

METHODS

Melanoma families and shared counseling protocol

The study was conducted on 208 Italian melanoma families that met the SIGU criteria and underwent clinical genetic counseling and testing.

The participating centers were San Martino Hospital in Genoa; Fondazione IRCSS—Istituto Nazionale dei Tumori and Istituto Europeo di Oncologia in Milan; Ospedale di Circolo-Università dell'Insubria in Varese; Ospedale Molinette in Turin; Istituto Oncologico Veneto in Padua; Policlinico Sant'Orsola-Malpighi in Bologna; Section of Medical Genetics, University of Florence; and Istituto Tumori in Bari.

Most of the families were referred for genetic counseling by local oncologists or dermatologists; a subset was referred by clinicians belonging to the Italian Melanoma Intergroup in Aviano, Pisa, and Naples.

The number of patients with melanoma, AAD for each patient, and number of patients with MPM in each family were recorded.

All of the families in the study were seen between 2000 and 2007. The subset of families seen before 2004, when the SIGU recommendations were drafted, was reassessed to check compliance with the SIGU criterion for clinical testing.

Written informed consent was obtained from all participants under ethics committee-approved protocols.

SIGU's shared eligibility criteria for clinical genetic counseling and testing, along with a flow chart

CAPSULE SUMMARY

- In Italy, clinical genetic testing for melanoma is currently offered to families with at least two affected members.
- We studied 204 Italian melanoma families that participated in clinical genetic counseling and testing, and found that 33% of the families overall, and 25% of those with just two affected members, carried mutations in *CDKN2A*, the primary melanoma susceptibility gene.
- Clinical genetic testing for melanoma in Italian families with just two affected members is justified in terms of the likelihood of identifying a *CDKN2A* mutation.

Abbreviations used:

AAD:	age at first melanoma diagnosis
CDK4:	<i>cyclin-dependent kinase 4</i>
CDKN2A:	<i>cyclin-dependent kinase inhibitor 2A</i>
GenoMEL:	International Melanoma Genetics Consortium
MPM:	multiple primary melanomas
SIGU:	Italian Society of Human Genetics

illustrating the clinical genetic counseling and testing process, are summarized in Fig 1.

Molecular analyses

Samples from Bari, Istituto Europeo di Oncologia in Milan, Turin, and Varese were sent to Genoa for testing, whereas families from the remaining centers were tested locally. The same standard protocol for testing was followed at all the centers that performed molecular analyses.

Genomic DNA was extracted from peripheral blood using standard methods. The *CDKN2A* coding region, including splice junctions, the 5'UTR, the intronic sequence described to contain the IVS2-105 A/G mutation,¹⁸ and exon 1 β was entirely sequenced, as was *CDK4* exon 2.

Detailed protocols of polymerase chain reaction and sequencing techniques have been previously described.⁸

Families were also tested for mutations in *CDK4* (exon 2). Evaluation of *CDK4* was restricted to exon 2 because no causal mutations have been identified outside of this exon. For mutation-positive families, the type of *CDKN2A* (exons 1 α , 2, and 3), *ARF* (exon 1 β), or *CDK4* (exon 2) mutation was recorded.

Approximately 90% of the families found to be negative for mutations in *CDKN2A* and *CDK4* by sequencing underwent multiplex ligation-dependent probe amplification analysis to investigate the presence of genomic rearrangements. The majority (70%) were tested in Florence and Padua¹⁹; 20% were tested in Genoa using the SALSA multiplex ligation-dependent probe amplification P024B 9p21 *CDKN2A/2B* kit (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. The remaining 10% was not tested as DNA was not available or not sufficient.

Statistical analyses

The nonparametric Wilcoxon-Mann-Whitney test was used to test the hypothesis of no difference in the distributions of the variables being compared. All statistical tests were two sided. Two-tailed *P* values of less than .05 were considered statistically significant.

RESULTS

Patients with melanoma

The 208 participating families included 513 patients with melanoma; 11 of 513 (2%) could not be confirmed, but two or more were confirmed in all families.

The median number of patients (both confirmed and not confirmed) per family was two, ranging from two to 11.

Mutation rates

Four noncoding *CDKN2A* variants with unknown functional significance were detected in as many families: IVS1+37G>C,¹³ 5'UTR -21C>T,²⁰ -56G>T and IVS2 -2A>G. As the pathogenicity of these variants is still unclear, these 4 families were not included in any further calculation.

A total of 33% (68/204) of the families harbored *CDKN2A* mutations. A single phenocopy was identified in a family with two affected members; one of these patients and an unaffected relative were found to carry the G23D mutation.¹¹ There was no significant difference in mutation rate between the families with one (34/112, 30%) and two (34/92, 37%) (*P* = .5744) tested cases. Further analyses were, therefore, conducted considering the families as a single series.

As shown in Fig 2, 14 different mutations were found in the 68 mutation-positive families; 50% of the mutations (*n* = 7) were observed only once; the remainder were seen in more than one family. The most frequent mutations were G101W (in 41 families, 60%), R24P (in 5 families, 7%), P48T (in 5 families, 7%), and E27X (in 4 families, 6%). Thus, although the G101W founder mutation accounted for roughly 60% of all the mutations identified (41/68 families), a further 23% is accounted for by other well-documented (E27X⁸, G23S⁹) or potential founder (P48T^{21,22}) mutations, or recurring mutations (R24P¹⁰).

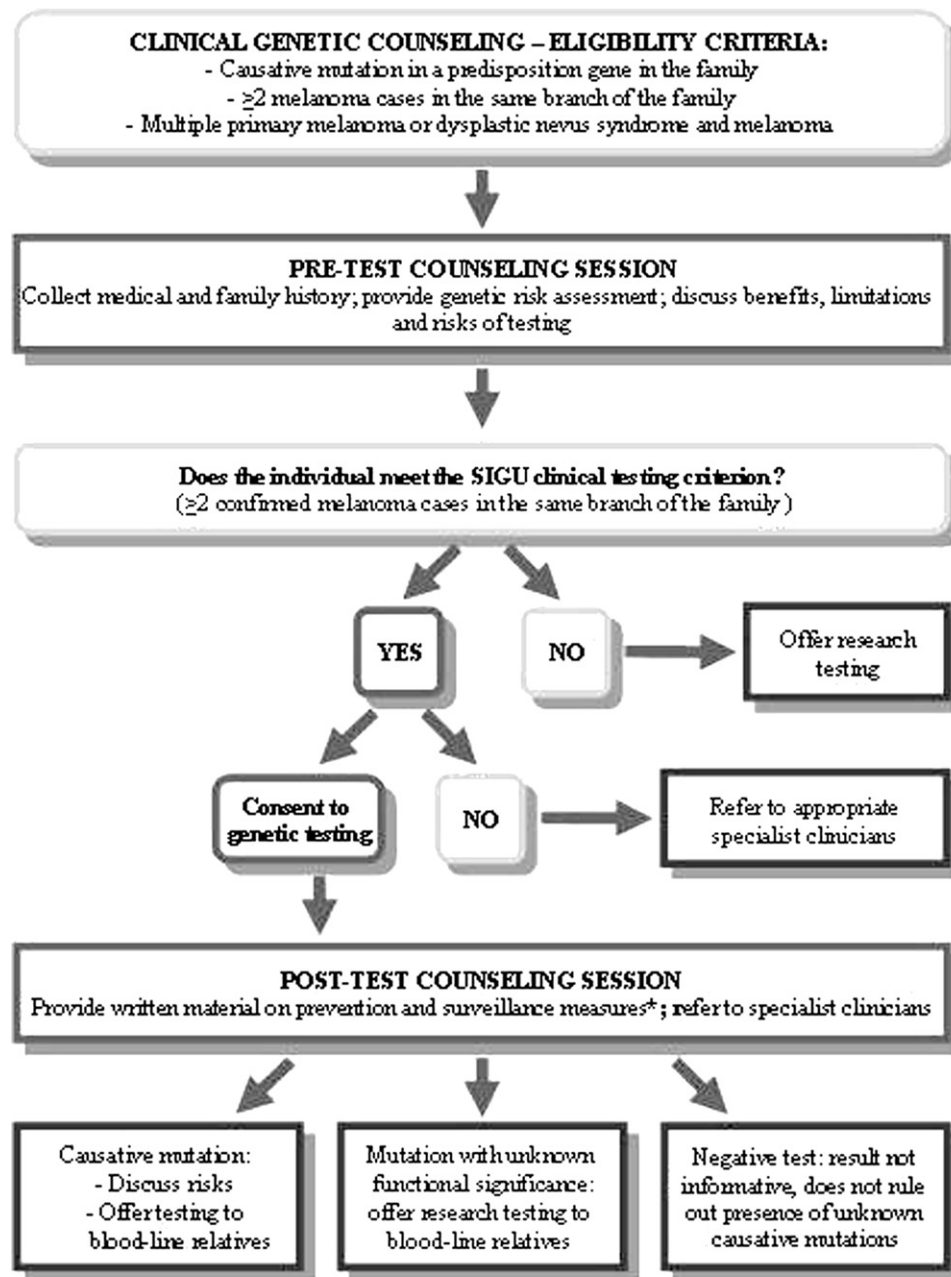
Three novel mutations, L94P, A86T, and c.407dupG, were identified among the cases and not in 200 controls.

No genomic alterations were detected by multiplex ligation-dependent probe amplification in the samples analyzed.

Mutation testing revealed that none of the families carried mutations in *CDK4*.

Mutation frequency according to number of affected cases per family, age at diagnosis, and presence of multiple primaries

As shown in Fig 3, *A*, the frequency of mutations increased significantly with the number of cases per family. In the 145 families with two affected



*Different approaches for follow-up of test-negative and -positive families are not recommended²³ but may be considered for carriers and non-carriers in families with causative mutations, including offering carriers additional encouragement to perform self skin examination, follow sun protection measures and undergo more frequent (i.e. six-monthly rather than yearly) clinical skin examinations.

Fig 1. Italian Society for Human Genetics' (SIGU) recommendations for clinical genetic counseling and testing for familial melanoma.

members the mutation frequency was 25% ($n = 36$), in the 41 families with 3 cases it was 46% ($n = 19$), and it reached 72% ($n = 13$) in the families with 4 or more cases. Overall, 54% of the families with 3 or more cases carried mutations in *CDKN2A*.

The median AAD was significantly different in patients from *CDKN2A*-positive families (42 years, range 14-78) compared with patients who belonged

to families with no mutations (49 years, range 11-93) ($P < .0001$).

Fig 3, B, shows the frequency of *CDKN2A* mutations in families with MPM cases. The frequency of mutations increased significantly with the number of patients with MPM in a family: 24% (34/144) of the families with no cases of MPM had *CDKN2A* mutations versus 49% (25/51) of the families with one

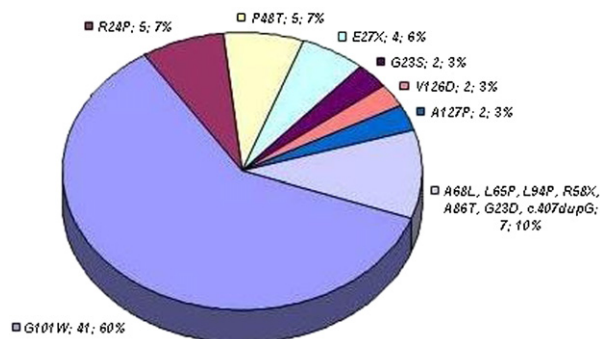


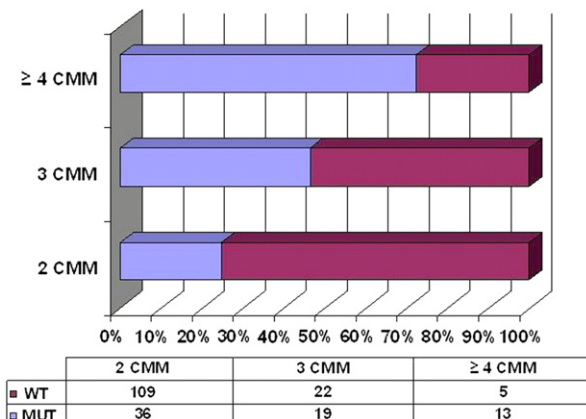
Fig 2. Cyclin-dependent kinase inhibitor 2A mutation distribution. Number and frequency of mutation-positive families are indicated after each mutation name.

patient with MPM, and 100% (9/9) of the families with two or more. Overall, 23% of the mutation-positive individuals (45 of 198) had developed MPM versus 9% (26 of 307) of the individuals who did not carry *CDKN2A* mutations ($P = .0002$).

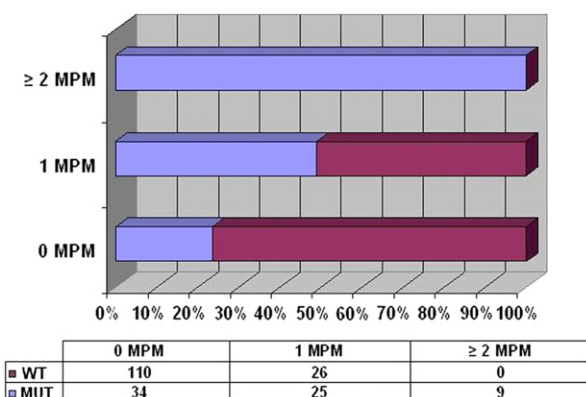
DISCUSSION

There has been much debate within GenoMEL as to whether clinical genetic testing for familial melanoma should be implemented. According to GenoMEL's first consensus statement²³ clinical genetic testing was premature, although rare exceptions were contemplated. In 2002 GenoMEL recognized that in countries such as Italy, where baseline melanoma incidence rates are low and founder mutations common, clinical genetic testing can encourage adherence to clinical recommendations among mutation carriers.²⁴ Indeed, Italy was one of the first countries where clinical genetic testing for familial melanoma was offered in medical or cancer genetics services. Because at the time there were no specific recommendations covering counseling and DNA testing for individuals perceived to be at risk, our clinical protocol was initially derived from published research and position papers.^{16,24-26} In 2004 SIGU drafted its recommendations,¹⁷ which have since formed the basis for access to genetic counseling and testing in Italy, and constitute the shared protocol adopted by all medical and cancer genetics services in our country.

In this study we sought to determine the frequency of *CDKN2A* and *CDK4* mutations in melanoma families counseled and tested in accordance with the SIGU recommendations in different Italian regions, and thus to verify whether compliance with these recommendations results in testing of individuals with a reasonable chance of carrying a *CDKN2A* mutation. The importance of establishing this mutation rate in our country is in the fact that one of the main reasons why clinical genetic testing for



A



B

Fig 3. Cyclin-dependent kinase inhibitor 2A mutation frequency according to number of affected cases (A) and presence of multiple primary melanomas (MPM) (B) in family. CMM, cutaneous malignant melanoma; MUT, mutated; WT, wild-type.

melanoma has yet to be widely implemented is that mutation rates in melanoma families vary widely among countries.²⁷

Because all the participating centers follow the same, nationally shared protocol, the pool of families studied was uniformly selected.

As expected, given that very few melanoma families worldwide have been reported to harbor mutations in *CDK4*,²⁸⁻³¹ and only one in Italy,¹⁵ none of our families harbored mutations in *CDK4*. Conversely, 33% of the families overall carried mutations in *CDKN2A*. One phenocopy was identified in a family, but when we compared the mutation rate of families with one and families with two tested melanoma cases we found no significant difference, as expected in a country of low incidence such as Italy where the likelihood of chance clustering of cases in a family is low.

Families with two cases accounted for 71% (145/204) of our entire sample and for 52% (36/68) of the families that carried *CDKN2A* mutations; thus

the *CDKN2A* mutation frequency in families with two affected members was 25%. These findings indicate that if a more conservative criterion for access to testing were adopted in Italy, such as the presence of at least 3 patients in the family, a substantial subset of families with *CDKN2A* mutations would not be identified. Indeed, the importance of including the geographic location in the assessment of candidacy for genetic counseling was recently stressed in a review on this topic.³²

Interestingly, the mutation rate we observed in the families with 3 or more melanoma cases (54%) was similar to the rate seen among the European families in the GenoMEL study,¹⁰ which, however, met a more stringent selection criterion (presence of at least 3 affected members).

The G101W founder mutation, the most common mutation observed to date in families worldwide,¹⁰ accounted for approximately 60% of the mutations detected in our pool of families, and another 23% of the mutations identified were known as possible founder mutations, or recurring mutations. Thus, founder or recurrent mutations seem to underlie susceptibility in the majority of familial melanoma cases in Italy, and may explain in part the high frequency of *CDKN2A* mutations in our two-case families.

We detected no genomic rearrangements, confirming that these alterations are a rare cause of melanoma susceptibility.^{19,33,34}

Patients from *CDKN2A* mutation-positive families had a significantly younger median AAD compared with patients who belonged to families with no mutations (42 vs 49 years, $P < .0001$), who in turn had a significantly earlier AAD compared with the median age at onset in the general Italian melanoma population (59 years).³⁵

In general, familial melanoma cases appear to have an earlier age at diagnosis than nonfamilial cases, and the incidence of *CDKN2A* mutations is higher in families with an early age at onset.^{13,23,36} In addition, the first tumor in patients with multiple melanoma tends to develop earlier than in patients with a single melanoma.³⁷ The early AAD observed in the *CDKN2A* mutation-negative families may be explained by the impact of other shared predisposing factors and as yet unknown susceptibility genes. Among our mutation-positive individuals, 23% had developed MPM versus 9% of those who did not carry *CDKN2A* mutations ($P = .0002$). The frequency of mutations increased significantly with the number of patients with MPM in the family and reached 100% in the families with two or more MPM, confirming that the number of cases with MPM increases the likelihood of detecting a germline *CDKN2A* mutation in a family.^{10,37-39}

In a very recent hospital-based study of single primary melanoma and MPM,²⁰ we found that the frequency of *CDKN2A* mutations in MPM cases was 32.6%. The MPM cases had a 4-fold higher likelihood of carrying a *CDKN2A* mutation than the single primary melanoma cases (odds ratio = 4.27; 95% confidence interval 2.43-7.53), independent of a family history of the disease, which suggests that the SIGU recommendations may be modified in the future to include the presence of MPM as a criterion for candidacy to genetic testing.

The main limitation of this study was that we were unable to conduct separate analyses for each center to explore possible geographic variations: indeed, in some cases the number of families was too small to allow statistical significance. Furthermore, the ascertainment of families was not population based, so we cannot rule out that there may be other families that met the SIGU criterion for clinical testing and were not analyzed. However, as testing was conducted on families with two or more patients with melanoma—a broad criterion—one might infer that the likelihood of a finding a lower mutation rate if all the families that met the criterion had been tested is not high.

Overall, our findings confirm that Italian melanoma families have a high mutation rate, that many of these mutation-positive families harbor founder mutations, and that the availability of clinical testing for melanoma to families with just two affected members is justified in our country in terms of the likelihood of identifying a mutation.

To date, the SIGU recommendations have not included the presence of pancreatic cancer in the proband or in first-degree relatives among the criteria for access to genetic counseling for melanoma susceptibility, given that data on the risk of pancreatic cancer in melanoma families are only available for a single Italian region.^{8,40} By continuing to study the families seen at our centers, we expect to be able to provide nationwide risk estimates.

REFERENCES

1. de Snoo FA, Hayward NK. Cutaneous melanoma susceptibility and progression genes. *Cancer Lett* 2005;230:153-86.
2. Gruis NA, van der Velden PA, Sandkuijl LA, Prins DE, Weaver-Feldhaus J, Kamb A, et al. Homozygotes for *CDKN2A* (p16) germline mutation in Dutch familial melanoma kindreds. *Nat Genet* 1995;10:351-3.
3. Borg A, Johannsson U, Johannsson O, Håkansson S, Westerdahl J, Mäsback A, et al. Novel germline p16 mutation in familial malignant melanoma in southern Sweden. *Cancer Res* 1996; 56:2497-500.
4. Ciotti P, Struwing JP, Mantelli M, Chompret A, Avril MF, Santi PL, et al. A single genetic origin for the G101W *CDKN2A*

- mutation in 20 melanoma-prone families. *Am J Hum Genet* 2000;67:311-9.
5. Goldstein AM, Liu L, Shennan MG, Hogg D, Tucker MA, Struwing JP. A common founder for the *V126D CDKN2A* mutation in seven North American melanoma prone families. *Br J Cancer* 2001;85:527-30.
 6. Hashemi J, Bendahl PO, Sandberg T, Platz A, Linder S, Stierner U, et al. Haplotype analysis and age estimation of the *113insR CDKN2A* founder mutation in Swedish melanoma families. *Genes Chromosomes Cancer* 2001;31:107-16.
 7. Jakobson E, Eisenberg S, Isacson R, Halle D, Levy-Lahad E, Catane R, et al. A single Mediterranean, possibly Jewish, origin for the Val59Gly *CDKN2A* mutation in four melanoma-prone families. *Eur J Hum Genet* 2003;11:288-96.
 8. Ghiorzo P, Gargiulo S, Pastorino L, Nasti S, Cusano R, Bruno W, et al. *E27X*, a novel *CDKN2A* germ line mutation, on p16 and *p14ARF* expression in Italian melanoma families displaying pancreatic cancer and neuroblastoma. *Hum Mol Genet* 2006;15:2682-9.
 9. Gensini F, Sestini R, Piazzini M, Vignoli M, Chiarugi A, Brandani P, et al. The *p.G23S CDKN2A* founder mutation in high-risk melanoma families from Central Italy. *Melanoma Res* 2007;17:387-92.
 10. Goldstein AM, Chan M, Harland M, Gillanders EM, Hayward NK, Avril MF, et al. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res* 2006;66:9818-28.
 11. Fagnoli MC, Chimenti S, Keller G, Soyer HP, Dal Pozzo V, Höfler H, et al. *CDKN2a/p16INK4a* mutations and lack of p19ARF involvement in familial melanoma kindreds. *J Invest Dermatol* 1998;111:1202-6.
 12. Ghiorzo P, Ciotti P, Mantelli M, Heouaine A, Queirolo P, Rainero ML, et al. Characterization of Ligurian melanoma families and risk of occurrence of other neoplasia. *Int J Cancer* 1999;83:441-8.
 13. Della Torre G, Pasini B, Frigerio S, Donghi R, Rovini D, Delia D, et al. *CDKN2A* and *CDK4* mutation analysis in Italian melanoma-prone families: functional characterization of a novel *CDKN2A* germ line mutation. *Br J Cancer* 2001;85:836-44.
 14. Mantelli M, Pastorino L, Ghiorzo P, Barile M, Bruno W, Gargiulo S, et al. Early onset may predict *G101W CDKN2A* founder mutation carrier status in Ligurian melanoma patients. *Melanoma Res* 2004;14:443-8.
 15. Majore S, De Simone P, Crisi A, Eibenschutz L, Binni F, Antigoni I, et al. *CDKN2A/CDK4* molecular study on 155 Italian subjects with familial and/or primary multiple melanoma. *Pigment Cell Melanoma Res* 2008;21:209-11.
 16. Bianchi-Scarrà G, Genuardi M, Pasini B, Tibiletti MG, Varesco L. Condizioni minime per la consulenza genetica in oncologia: Gruppo SIGU-ONC; 2000. Available from: URL:<http://www.sigu.net>. Accessed October 8, 2008.
 17. Bianchi-Scarrà G, Grammatico P, Genuardi M, Pasini B. Raccomandazioni per consulenza e test genetico nel melanoma ereditario: Gruppo SIGU-ONC; 2004. Available from: URL:<http://www.sigu.net>. Accessed October 8, 2008.
 18. Harland M, Mistry S, Bishop DT, Newton Bishop JA. A deep intronic mutation in *CDKN2A* is associated with disease in a subset of melanoma pedigrees. *Hum Mol Genet* 2001;10:2679-86.
 19. Vignoli M, Scaini MC, Ghiorzo P, Sestini R, Bruno W, Menin C, et al. Genomic rearrangements of the *CDKN2A* locus are infrequent in Italian malignant melanoma families without evidence of *CDKN2A/CDK4* point mutations. *Melanoma Res* 2008;18:431-7.
 20. Pastorino L, Bonelli L, Ghiorzo P, Queirolo P, Battistuzzi L, Balleari E, et al. *CDKN2A* mutations and *MC1R* variants in Italian patients with single or multiple primary melanoma. *Pigment Cell Melanoma Res* 2008;21:700-9.
 21. Huber J, Ramos ES. The P48T germline mutation and polymorphism in the *CDKN2A* gene of patients with melanoma. *Braz J Med Biol Res* 2006;39:237-41.
 22. Széll M, Balogh K, Dobozy A, Kemény L, Oláh J. First detection of the melanoma-predisposing proline-48-threonine mutation of p16 in Hungarians: was there a common founder either in Italy or in Hungary? *Melanoma Res* 2007;17:251-4.
 23. Kefford RF, Newton Bishop JA, Bergman W, Tucker MA. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: a consensus statement of the melanoma genetics consortium. *J Clin Oncol* 1999;17:3245-51.
 24. Kefford R, Bishop JN, Tucker M, Bressac-de Paillerets B, Bianchi-Scarrà G, Bergman W, et al. Genetic testing for melanoma. *Lancet Oncol* 2002;3:653-4.
 25. Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility; adopted on February 20, 1996. *J Clin Oncol* 1996;14:1730-6.
 26. Olopade OI, Offit K, Garber JE. Genetic testing for susceptibility to cancer: task force on cancer genetics education. *JAMA* 1998;279:1612-3.
 27. Gerstenblith MR, Goldstein AM, Tucker MA, Fraser MC. Genetic testing for melanoma predisposition: current challenges. *Cancer Nurs* 2007;30:452-9.
 28. Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, et al. Germline mutations in the *p16INK4a* binding domain of *CDK4* in familial melanoma. *Nat Genet* 1996;12:97-9.
 29. Soufir N, Avril MF, Chompret A, Demenais F, Bombléd J, Spatz A, et al. Prevalence of *p16* and *CDK4* germline mutations in 48 melanoma-prone families in France: the French familial melanoma study group. *Hum Mol Genet* 1998;7:209-16.
 30. Molven A, Grimstvedt MB, Steine SJ, Harland M, Avril MF, Hayward NK, et al. A large Norwegian family with inherited malignant melanoma, multiple atypical nevi, and *CDK4* mutation. *Genes Chromosomes Cancer* 2005;44:10-8.
 31. Pjanova D, Engele L, Randerson-Moor JA, Harland M, Bishop DT, Newton Bishop JA, et al. *CDKN2A* and *CDK4* variants in Latvian melanoma patients: analysis of a clinic-based population. *Melanoma Res* 2007;17:185-91.
 32. Leachman SA, Carucci J, Kohlmann W, Banks K, Asgari M, Bergman W, et al. Evidence-based selection of familial melanoma patients for genetic assessment. *J Am Acad Dermatol*. In press.
 33. Lesueur F, de Lichy M, Barrois M, Durand G, Bombléd J, Avril MF, et al. The contribution of large genomic deletions at the *CDKN2A* locus to the burden of familial melanoma. *Br J Cancer* 2008;99:364-70.
 34. Mistry SH, Taylor C, Randerson-Moor JA, Harland M, Turner F, Barrett JH, et al. Prevalence of 9p21 deletions in UK melanoma families. *Genes Chromosomes Cancer* 2005;44:292-300.
 35. Crocetti E, Capocaccia R, Casella C, De Lisi V, Ferretti S, Foca F, et al. Associazione Italiana Registri Tumori: I Tumori in Italia Rapporto 2006. Incidenza, mortalità e stime. *Epidemiol Prev* 2006;30:1-147.
 36. Holland EA, Schmid H, Kefford RF, Mann GJ. *CDKN2A (P16/INK4a)* and *CDK4* mutation analysis in 131 Australian melanoma probands: effect of family history and multiple

- primary melanomas. *Genes Chromosomes Cancer* 1999;25:339-48.
37. Monzon J, Liu L, Brill H, Goldstein AM, Tucker MA, From L, et al. *CDKN2A* mutations in multiple primary melanomas. *N Engl J Med* 1998;338:879-87.
38. Mantelli M, Barile M, Ciotti P, Ghorzo P, Lantieri F, Pastorino L, et al. High prevalence of the G101W germline mutation in the *CDKN2A (P16(ink4a))* gene in 62 Italian malignant melanoma families. *Am J Med Genet* 2002;107:214-21.
39. Puig S, Malvey J, Badenas C, Ruiz A, Jimenez D, Cuellar F, et al. Role of the *CDKN2A* locus in patients with multiple primary melanomas. *J Clin Oncol* 2005;23:3043-51.
40. Ghorzo P, Pastorino L, Bonelli L, Cusano R, Nicora A, Zupo S, et al. *INK4/ARF* germline alterations in pancreatic cancer patients. *Ann Oncol* 2004;15:70-8.