

Familial Melanoma; CDKN2A and Beyond

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The most common hereditary melanoma susceptibility disorder is the familial atypical multiple mole-melanoma (FAMMM) syndrome. FAMMM is regarded as an ideal natural model to study the very complex pathologic mechanism of melanoma. In 1994, cloning of the melanoma susceptibility gene CDKN2A was thought to give answers to many questions on genotype-phenotype correlations in familial melanoma. Today, 4 y later, germline mutations cosegregate with melanoma in only 40%–50%

of the families predisposed to this disease. The hunt for genes and modifying genes is on again. Through the years the very well-characterized Dutch FAMMM families have proven to be valuable study subjects in melanoma research. This paper describes over 10 y of melanoma research illustrated by research performed in the Dutch FAMMM families. Key words: CDKN2A/hereditary melanoma/melanocortin 1 receptor. Journal of Investigative Dermatology Symposium Proceedings 4:50–54, 1999

FAMMM; LINKAGE STUDIES ON CHROMOSOME 1P

The familial occurrence of cutaneous malignant melanoma was already recognized in 1820 by Norris (1820). The association of familial melanoma with inherited atypical nevi was originally described as the BK mole syndrome (Clark *et al*, 1978), but became more generally known as the dysplastic nevus (DNS) syndrome and the familial atypical multiple mole-melanoma (FAMMM) syndrome. It has been estimated that 8%–12% of melanomas occur in the familial setting (Greene and Fraumeni, 1979).

In 1982, the Pigmented Lesion Clinic at the Leiden Department of Dermatology was established to facilitate studies on familial melanoma. Shortly thereafter, evidence of familial occurrence of melanoma was found in 18 melanoma patients and today, more than a hundred families have been registered. Ten of these families have been very well documented with respect to number of melanoma and atypical nevi validating the diagnosis of the FAMMM syndrome (Bergman *et al*, 1992). Most Dutch FAMMM families are from the Leiden region and live in two towns within a distance of 20 km from the hospital. The population in these regions has been stationary for several generations due to their orthodox religion and strong family ties.

In 1983, the first linkage analysis in melanoma families was undertaken by Greene *et al* (1983). Using the combination of melanoma and atypical nevi as the disease phenotype, nonsignificant evidence for linkage between a melanoma/atypical nevus predisposition gene and the Rhesus blood group locus on the short arm of chromosome 1 was supported. In 1989, the same group localized the gene to a more distal chromosome 1 region between the DNA markers D1S47 and PND (Bale *et al*, 1989). Coinciding with this report we published data excluding all of the short arm of chromosome 1 in six Dutch FAMMM families (van Haeringen *et al*, 1989), including the D1S47 and PND marker region (Gruis *et al*, 1990). No supporting evidence for a melanoma and/or nevus susceptibility locus in the latter region was

provided by marker typing in melanoma families from North America (Cannon-Albright *et al*, 1990) and Australia (Kefford *et al*, 1991; Nancarrow *et al*, 1992).

CDKN2A, A MELANOMA SUSCEPTIBILITY GENE ON CHROMOSOME 9P

In 1992, Cannon-Albright *et al* (1992) provided highly significant evidence for a melanoma susceptibility locus in the chromosomal region 9p13-p22. In their analysis, only patients with melanoma were considered as affected, ignoring the occurrence of atypical nevi. Two-point and multipoint linkage analysis in Dutch (Gruis *et al*, 1993, 1995a; Bergman *et al*, 1994), Australian (Nancarrow *et al*, 1993), and British (MacGeoch *et al*, 1994) FAMMM kindreds confirmed the localization of the melanoma locus. Analysis of the American families earlier found to be linked to chromosome 1, now suggested that some of them are linked to chromosome 9 as well (Goldstein *et al*, 1996), although a number of families continues to show linkage to chromosome 1.

In a very short period of time, an interesting candidate gene for melanoma susceptibility was cloned from the 9p21 region (Kamb *et al*, 1994b; Nobori *et al*, 1994). The gene product had been earlier identified as the cyclin-dependent kinase inhibitor, p16 (Serrano *et al*, 1993). The p16 gene has been variably called MTS1, INK4A, and CDK4I; nowadays CDKN2A is the most accepted nomenclature. The CDKN2A gene is a key player in cell cycle regulation (Serrano *et al*, 1993). The gene encodes the p16 protein that binds and inhibits cyclin dependent kinase (CDK) 4 and 6, an inhibition that prevents complex formation of CDK4 with cyclin D1. The CDK4/cyclin D complex normally supports the transition from G1 to S phase in the cell cycle by phosphorylating and inactivating the retinoblastoma gene product. Homozygous deletions and mutations of CDKN2A in cell lines and primary tumors of several tumor types support a general tumor suppressor function for the CDKN2A gene (Kamb *et al*, 1994a; Weaver-Feldhaus *et al*, 1994; Gruis *et al*, 1995c; Liu L *et al*, 1995).

CDKN2A, THE MAJOR MELANOMA GENE

Support for the role of the CDKN2A in melanoma predisposition was additionally provided by the identification of germline mutations in affected members of melanoma kindreds all over the world (Gruis *et al*,

Manuscript received December 10, 1998; revised March 2, 1999; accepted for publication March 9, 1999.

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1995b; Hussussian *et al.* 1994; Liu Q *et al.* 1995; Harland *et al.* 1997; Pollock *et al.* 1998). Germline mutations in CDKN2A are typically point mutations, insertions, or small intragenic deletions within the first or second exon and are present in approximately 40%–50% of the 9p-linked melanoma families studied (Foulkes *et al.* 1997).

In most of the Dutch FAMMM families a 19 bp germline deletion (p16-Leiden) in exon 2 of the CDKN2A gene was observed in melanoma patients (Gruis *et al.* 1995b). The deletion introduces a frameshift resulting in a truncated p16 protein. Consequently, two of the four ankyrin domains of p16, by which p16 binds to CDK4, are disrupted by the deletion. All families in which the deletion has been observed came from the same geographic area of the Netherlands and carried the same 9p21 haplotype. A common founder for these families was observed in the year 1707 (Hille *et al.* 1998).

Of potential importance regarding the role of p16 in the development of melanoma was the discovery of two individuals within one of the Dutch melanoma kindreds with germline deletions in both copies of CDKN2A (Gruis *et al.* 1995b). Surprisingly, one of these human “knock-outs” did not develop melanoma prior to her death at the age of 55 from an adenocarcinoma. The presence of three very mildly atypical nevi was the only sign possibly related to FAMMM. Her progeny had the classic FAMMM phenotype, with melanomas and atypical nevi present in two of her three children. On the contrary, the other homozygous individual developed atypical moles in very large numbers at the age of 11. Already at the age of 15, he developed a melanoma *in situ* and an invasive melanoma. Our observation of normal development in the absence of functional p16 implies that p16 is not sufficient to develop melanoma. Either other endogenous or exogenous factors or the presence of functionally redundant genes seems to be required for phenotypic expression.

ADDITIONAL CELL CYCLE REGULATORS AS CANDIDATE GENES FOR MELANOMA

In conjunction with the cloning of the CDKN2A gene from the 9p21 region, a gene with 93% sequence homology to the CDKN2A gene was cloned 30 kb downstream of CDKN2A (Kamb *et al.* 1994a). This CDKN2B gene encodes p15; another inhibitor of CDK4. CDKN2B is often found to be codeleted together with the CDKN2A gene in many tumor cell lines, including melanoma (Suzuki *et al.* 1995; Stadler and Olopade, 1996; Jadayel *et al.* 1997; Hamada *et al.* 1998; Walker *et al.* 1998). The latter suggests that p15 may be a tumor suppressor as well. Ectopic expression of p15 inhibits growth of tumor-derived cell lines, although lack of p15 mutations in tumor cell lines and in 9p21-linked melanoma kindreds suggests a role for p15 in growth regulation, but a limited role for p15 in tumor progression (Liu *et al.* 1997; Stone *et al.* 1995).

P16 regulates passage through the G1 checkpoint of the cell cycle by CDK4/cyclinD1 and CDK6/cyclinD1 kinases that phosphorylate the retinoblastoma protein. This negative regulatory pathway suggests several other candidates for examination in not 9p-linked melanoma kindreds. Today, in two unrelated melanoma families who do not carry p16 germline mutations, an Arg24Cys mutation in the CDK4 gene has been observed that averts binding of the p16 protein to CDK4 (Zuo *et al.* 1996). In one other 9p-linked melanoma kindred a Arg24His mutation was observed (Soufir *et al.* 1998). Despite the strong evidence that CDK4 can be considered a second familial melanoma gene, the very low frequency of CDK4 mutations in melanoma kindreds suggests that additional genes play a role in the development of familial melanoma.

New light may be shed on the role of the CDKN2A gene in tumor development by the recent finding of a second protein product of the CDKN2A gene (p14ARF, p19ARF in the mouse), which originates from an unrelated exon of CDKN2A (exon 1 β) spliced onto exon 2 in an alternate reading frame. P16 is a recognized tumor suppressor that induces a G1 cell cycle arrest by inhibiting the phosphorylation of the retinoblastoma protein by the cyclin-dependent kinases, CDK4 and CDK6. The product of the human CDKN2A β transcript, p14ARF, activates a p53 response that manifests in elevated levels of MDM2 and p21(CIP1) and in cell cycle arrest in both G1 and G2/M

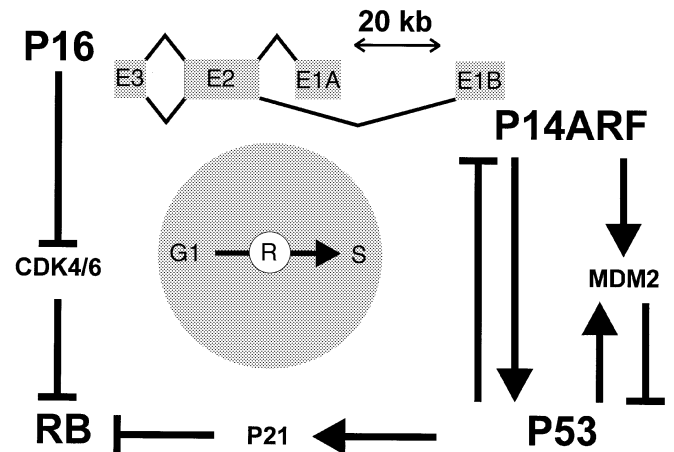


Figure 1. Schematic representation of the role of the p16 and p14ARF proteins in cell cycle regulation. P16 and p14ARF restrain cell growth by affecting the functions, the retinoblastoma protein and p53, respectively.

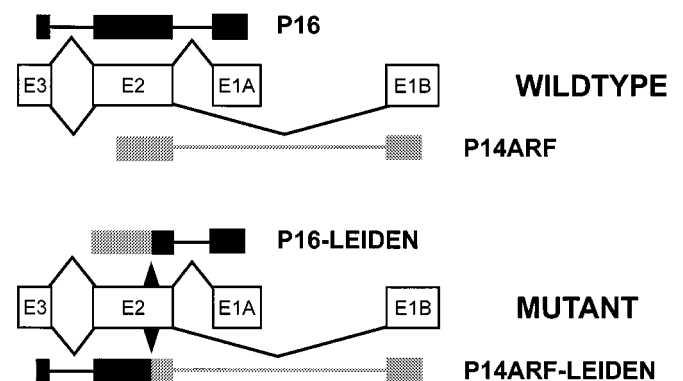


Figure 2. Schematic representation of the different CDKN2A and p14ARF transcripts obtained in the wild-type situation and in the case of a 19 bp deletion of exon 2 (p16-Leiden and p14ARF-Leiden). At the position of the deletion in exon 2 (as depicted by a black triangle) the reading frame is changed for both the CDKN2A and the p14ARF transcript. The p14ARF transcript is followed by the CDKN2A transcript at the point of the deletion.

(Kamijo *et al.* 1997, 1998; Stott *et al.* 1998) (Fig 1). P14ARF acts by binding directly to both p53 and MDM2, resulting in the stabilization of p53 and MDM2. Conversely, p53 negatively regulates p14ARF expression and there is an inverse correlation between p14ARF expression and p53 function in human tumor cell lines. p14ARF expression, however, is not involved in the response to DNA damage. Binding of the p14ARF N-terminal domain to p53 (encoded by CDKN2A exon 1 β) seems to be necessary and sufficient to induce cell cycle arrest (Quelle *et al.* 1997). These results place p14ARF in an independent pathway upstream of p53, and imply that CDKN2A encodes two proteins that are involved in tumor suppression.

Until now, no germline mutations have been observed in the first exon (exon 1 β) of p14ARF in melanoma families (FitzGerald *et al.* 1996; Liu *et al.* 1997), although germline mutations observed in CDKN2A exon 2 might effect both p16 and p14ARF function due to the dual utilization this exon (FitzGerald *et al.* 1996). Frameshift mutations have the potential to produce chimeric proteins. A potential of chimeric protein productions is nicely illustrated in the Dutch FAMMM families in which a 19 bp deletion in exon 2 of CDKN2A leads to a frameshift yielding a transcript encoding the N-terminal half of p14ARF fused to the C-terminal half of p16 (Fig 2). The generated fusion protein shows no binding capacity to CDK4 or CDK6 (Dr. G. Peters, personal communication). Data on the p14ARF-related function of the protein are required.

MODIFICATION OF MELANOMA RISK BY POLYMORPHISMS IN THE MELANOCYTE-STIMULATING HORMONE RECEPTOR GENE

Whereas one of the major risk factors for skin cancer, ultraviolet radiation, is environmental (Armstrong and Kricger, 1996), the other major determinant of skin cancer, namely the pigmentary status of the individual, is definitely genetic (Armstrong and Kricger, 1996). Murine genetics has proven to be a powerful tool to study the genetics of pigmentation. The identification of the murine extension locus as the human melanocortin 1 receptor (MC1R) (Mountjoy *et al*, 1992; Robbins *et al*, 1993) stimulated human studies of the cutaneous response to ultraviolet radiation. MC1R is a seven transmembrane domain G protein-coupled receptor encoded by the MC1R gene on chromosome 16q24.3 (Gantz *et al*, 1994). The natural agonist of the MC1R is α -melanocyte stimulating hormone that acts via MC1R to increase the synthesis of eumelanin (brown/black pigment) in melanocytes (Hunt *et al*, 1995).

In analogy with, for example, mice, foxes, and cattle, where variants of MC1R are associated with various coat colors (Robbins *et al*, 1993; Joerg *et al*, 1996; Vage *et al*, 1997), in humans, variants in MC1R have been linked with sun-sensitive skin types (Valverde *et al*, 1995) and in addition melanoma susceptibility (Valverde *et al*, 1996). Valverde *et al* (1995) showed that polymorphisms within the MC1R coding region turned out to be present in 80% of individuals of a predominantly British population with red hair and/or fair skin that tans poorly. Their results suggested that MC1R is a control point in the regulation of pigmentation phenotype and that amino acid variations in the MC1R protein are associated with a poor tanning response. A major drawback of this study was that individuals were selected from the extremes of hair color or skin type, providing no adequate basis for risk calculations or mode of inheritance studies on pigmentary phenotype.

In a consecutive series of Irish volunteers the contribution of MC1R variants to pigmentation was furthermore substantiated (Smith *et al*, 1998). Seventy-five per cent of the study group contained a MC1R sequence variant in the gene, with 30% containing two variants. MC1R variants seemed to be associated with red-haired individuals in this Irish population in which three variants in particular, Arg151Cys, Arg160Trp, and Asp294His, showed over-representation. The very same variants were also over-represented in individuals with fair skin. The presence of one of these variants as homozygous or compound heterozygous in red-haired individuals suggests a possible Mendelian recessive inheritance for red hair. Follow up of the Asp294His variant in 57 subjects with nonmelanoma skin cancer from a UK population showed a significantly increased frequency of this variant in this study group. Again, one should realize that in this study design, the MC1R gene has not been checked for all possible variants present. In the cancer study group, individuals were only screened for the presence of the Asp294His variant without analysis for additional variants.

The association between MC1R coding region variations Arg151Cys, Arg160Trp and Asp294His and red hair was confirmed in a study of Australian monozygotic and dizygotic twins (Box *et al*, 1997). In addition to the study of Valverde *et al* (1995), the variant Val60Leu was more apparent in fair/blonde and light brown hair colors. Although no variant by itself was associated with any skin type, studies on MC1R genotypes showed a strong association with hair color and skin type when allowing for any two MC1R variants in the genotype. Both studies clearly demonstrate that human red hair color is associated with variant MC1R alleles, although discordance for red hair color in dizygotic twins, who both share MC1R haplotypes, suggests that variants in MC1R are necessary but not sufficient for red hair.

The link of MC1R variants with sun-sensitive skin types suggests the MC1R gene to be a susceptibility candidate for melanoma. Valverde *et al* (1996) compared the frequency of earlier described MC1R gene variants (Valverde *et al*, 1995) in 43 unrelated melanoma patients and 44 controls. MC1R variants were more common in cases than controls, resulting in a relative risk of 3.91 for melanoma (95% confidence interval 1.48–10.35). Of note, the Asp84Glu variant turned out to be present in melanoma cases, although the contribution of variant MC1R

alleles in melanoma cases was largely independent of skin type, suggesting a more causal role for MC1R in melanoma development.

Associations of MC1R variants with skin type, hair color, and melanoma susceptibility were followed up in a large case-control study of 306 melanoma cases and 190 controls (Ichii-Jones *et al*, 1998). Unfortunately, only the frequency of the Val92Met, Asp294His, and Asp84Glu alleles has been determined. The allele frequencies of the three variants observed in melanoma cases were not different from the frequencies experienced in the control population. Within the cases and control group, the frequency of studied alleles turned out to be similar in skin type I and II individuals compared with individuals with skin types III and IV. In conjunction with earlier studies (Valverde *et al*, 1995; Box *et al*, 1997), an association was found between skin type and the presence of one or two variant alleles in the genotype of control individuals. Unexpectedly, although not significantly proven, the presence of one or more variant alleles in the genotype in cases was associated with tanning rather than burning of the skin. The earlier observed association of MC1R variants and hair type could not be observed in any of the variants under study in either cases or controls. Therefore, in this study the variants Val92Met, Asp294His, and Asp84Glu were not considered to be major determinants of skin type or melanoma risk.

At the moment, the Leiden Skin Cancer Study has started in which we explore the relationship between sun exposure, pigmentary traits, and MC1R variants in a large case control study, including pigmented and nonpigmented skin cancer. In addition, MC1R variant studies in Dutch FAMMM families are on their way to explore the modifying effect of MC1R variants on melanoma risk.

The discrepancies between the studies might relate to population-based differences in allele frequencies, the numbers of controls used, or selection of the controls. Studies of the functional significance of the various alleles might shed light on the real involvement of MC1R variants in pigmentation phenotype or melanoma development. The position of the Val92Met variant in the second trans membrane domain of the receptor was thought to alter the α -helix structure of the second transmembrane domain; however, the finding that the receptor ligand α -melanocyte stimulating hormone (α -MSH) has a slightly reduced affinity (5% less) for its receptor (Xu, 1996) suggests that the Val92Met variant is a neutral polymorphism that does not affect the relative amounts of eumelanin and pheomelanin produced. Both the Val92Met and the Asp84Glu variant have been expressed in heterologous cells to measure changes in intracellular cAMP in response to α -MSH. Again, neither of these variants show significant changes from the wild-type response (Koppula *et al*, 1997). The Arg151Cys variant seems more obviously to affect receptor functioning. This variant is located in the second intracellular domain in a consensus sequence for cAMP-dependent protein kinase. This change might block phosphorylation and hence receptor signaling.

GENETICS OF FAMILIAL MELANOMA, HOW FAR HAVE WE COME?

Identification of germline mutations within the CDKN2A gene in melanoma families has been a considerable step forward in the field of melanoma genetics. Despite this major discovery, CDKN2A seems to be related to melanoma in only 40% of 9p-linked melanoma families. This relatively small proportion suggests that mutations outside the coding region or other nearby located genes are responsible for melanoma susceptibility. Loss of coordinate expression of the CDKN2A and p14ARF transcripts may be an alternative genetic basis for melanoma susceptibility in certain 9p-linked kindreds. Rizos *et al* (1997) analyzed allele-specific expression levels of both the CDKN2A and the p14ARF transcripts in B-lymphoblastoid cells from 18 members of hereditary melanoma kindreds, including four unrelated control individuals. In 15 of the 18 individuals examined, steady-state levels of each transcript either originated equally from each parental chromosome, or one parental chromosome was dominant for both transcripts. In three affected members of two 9p-linked kindreds without exonic CDKN2A mutations, however, this pattern of coordinate expression was disrupted. In these individuals there was underexpression of the

p14ARF transcript, relative to the CDKN2A transcript, from the chromosome segregating with disease susceptibility. Methylation of the 5' CpG island of the CDKN2A tumor suppressor gene has proven to be a commonly used mechanism of inactivation of this cell cycle regulatory gene in many tumor types (Herman *et al.* 1995). Although methylation-associated gene silencing seems not to represent a common mechanism for CDKN2A inactivation in sporadic melanoma (Gonzalzo *et al.* 1997).

Apart from the absence of germline mutations in 9p-linked families, the relationship between the CDKN2A gene and the second clinical phenotype of the FAMMM syndrome, atypical nevi (AN) remains unresolved. Inclusion of patients with over 10 AN and melanoma in the linkage analysis in Dutch FAMMM families increased the 9p-linked lod score (Gruis *et al.* 1993). Lower numbers of AN diminished the lod score. In addition to these results, the presence of many FAMMM family members in Dutch as well as American families with numerous AN without mutations in the CDKN2A gene, implies the existence of additional genes for the predisposition and development of AN. Identification of genomic regions that show increased sharing (identity by descent) by the nevus patients in the very stationary Dutch FAMMM families are now underway to localize and identify these genes.

Therefore, 6 y after the localization of a melanoma locus on chromosome 9p, a new field of research has opened up the search for additional melanoma genes, nevus genes, interactions between melanoma genes and modifying genes, and interactions between melanoma genes and environmental factors to explain the complex character of melanoma genetics.

We thank the Dutch Cancer Foundation (94-758) for generous support. NAG is a fellow of the Royal Netherlands Academy of Arts and Sciences. We are grateful to Dr. Marianne Berwick for critical reading of the manuscript.

REFERENCES

- Armstrong BK, Kricker A: Epidemiology of sun exposure and skin cancer. *Cancer Surv* 26:133-153, 1996
- Bale SJ, Dracopoli NC, Tucker MA, *et al.*: Mapping the gene for hereditary cutaneous malignant melanoma-dysplastic nevus to chromosome 1p. *N Engl J Med* 320:1367-1372, 1989
- Bergman W, Gruis NA, Frants RR: The Dutch FAMMM family material: clinical and genetic data. *Cytogenet Cell Genet* 59:161-164, 1992
- Bergman W, Gruis NA, Sandkuijl LA, Frants RR: Genetics of seven Dutch familial atypical multiple mole-melanoma syndrome families: a review of linkage results including chromosomes 1 and 9. *J Invest Dermatol* 103:122S-125S, 1994
- Box NF, Wyeth JR, O'Gorman LE, Martin NG, Sturm RA: Characterization of melanocyte stimulating hormone receptor variant alleles in twins with red hair. *Hum Mol Genet* 6:1891-1897, 1997
- Cannon-Albright LA, Goldgar DE, Wright EC, *et al.*: Evidence against the reported linkage of the cutaneous melanoma-dysplastic nevus syndrome locus to chromosome 1p36. *Am J Hum Genet* 46:912-918, 1990
- Cannon-Albright LA, Goldgar DE, Meyer LJ, *et al.*: Assignment of a locus for familial melanoma, MLM, to chromosome 9p13-p22. *Science* 258:1148-1152, 1992
- Clark WH, Reimer RR, Greene M, Ainsworth AM, Mastrangelo MJ: Origin of familial malignant melanomas from heritable melanocytic lesions. "The BK mole syndrome". *Arch Dermatol* 732:732-738, 1978
- FitzGerald MG, Harkin DP, Silva-Arrieta S, *et al.*: Prevalence of germ-line mutations in p16, p19ARF, and CDK4 in familial melanoma: analysis of a clinic-based population. *Proc Natl Acad Sci USA* 93:8541-8545, 1996
- Foulkes WD, Flanders TY, Pollock PM, Hayward NK: The CDKN2A (p16) gene and human cancer. *Mol Med* 3:5-20, 1997
- Gantz I, Yamada T, Tashiro T, Konda Y, Shimoto Y, Miwa H, Trent JM: Mapping of the gene encoding the melanocortin-1 (alpha-melanocyte stimulating hormone) receptor (MC1R) to human chromosome 16q24.3 by Fluorescence in situ hybridization. *Genomics* 19:394-395, 1994
- Goldstein AM, Goldin LR, Dracopoli NC, Clark WH Jr, Tucker MA: Two-locus linkage analysis of cutaneous malignant melanoma/dysplastic nevi. *Am J Hum Genet* 58:1050-1056, 1996
- Gonzalzo ML, Bender CM, You EH, *et al.*: Low frequency of p16/CDKN2A methylation in sporadic melanoma: comparative approaches for methylation analysis of primary tumors. *Cancer Res* 57:5336-5347, 1997
- Greene MH, Fraumeni JF: The hereditary variant of malignant melanoma. In: Clark WH, Goldman L, Mastrangelo M (eds). *Human Malignant Melanoma*. New York: Grune and Stratton, 1979, pp. 139-166
- Greene MH, Sanders RJ, Chu FC, Clark WH Jr, Elder DE, Cogan DG: The familial occurrence of cutaneous melanoma, intraocular melanoma, and the dysplastic nevus syndrome. *Am J Ophthalmol* 96:238-245, 1983
- Gruis NA, Bergman W, Frants RR: Locus for susceptibility to melanoma on chromosome 1p. *N Engl J Med* 322:853-854, 1990
- Gruis NA, Sandkuijl LA, Weber JL, van der Zee A, Borgstein AM, Bergman W, Frants RR: Linkage analysis in Dutch familial atypical multiple mole-melanoma (FAMMM) syndrome families. Effect of naevus count. *Melanoma Res* 3:271-277, 1993
- Gruis NA, Sandkuijl LA, van der Velden PA, Bergman W, Frants RR: CDKN2 explains part of the clinical phenotype in Dutch familial atypical multiple-mole melanoma (FAMMM) syndrome families. *Melanoma Res* 5:169-177, 1995a
- Gruis NA, van der Velden PA, Sandkuijl LA, *et al.*: Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. *Nat Genet* 10:351-353, 1995b
- Gruis NA, Weaver-Feldhaus J, Liu Q, *et al.*: Genetic evidence in melanoma and bladder cancers that p16 and p53 function in separate pathways of tumor suppression. *Am J Pathol* 146:1199-1206, 1995c
- van Haeringen A, Bergman W, Nelen MR, *et al.*: Exclusion of the dysplastic nevus syndrome (DNS) locus from the short arm of chromosome 1 by linkage studies in Dutch families. *Genomics* 5:61-64, 1989
- Hamada K, Kohno T, Kawanishi M, Ohwada S, Yokota J: Association of CDKN2A (p16) /CDKN2B (p15) alterations and homozygous chromosome arm 9p deletions in human lung carcinoma. *Genes Chromosomes Cancer* 22:232-240, 1998
- Harland M, Meloni R, Gruis N, *et al.*: Germline mutations of the CDKN2 gene in UK melanoma families. *Hum Mol Genet* 6:2061-2067, 1997
- Herman JG, Merlo A, Mao L, *et al.*: Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res* 55:4525-4530, 1995
- Hille ET, van Duijn E, Gruis NA, Rosendaal FR, Bergman W, Vandenbroucke JP: Excess cancer mortality in six Dutch pedigrees with the familial atypical multiple mole-melanoma syndrome from 1830 to. *J Invest Dermatol* 110:788-792, 1998
- Hunt G, Kyne S, Wakamatsu K, Ito S, Thody AJ: Nle4DPhe7 alpha-melanocyte-stimulating hormone increases the eumelanin: pheomelanin ratio in cultured human melanocytes. *J Invest Dermatol* 104:83-85, 1995
- Hussussian CJ, Struwing JP, Goldstein AM, *et al.*: Germline p16 mutations in familial melanoma. *Nat Genet* 8:15-21, 1994
- Ichii-Jones F, Lear JT, Heagerty AH, *et al.*: Susceptibility to melanoma: influence of skin type and polymorphism in the melanocyte stimulating hormone receptor gene. *J Invest Dermatol* 111:218-221, 1998
- Jadayel DM, Lukas J, Nacheva E, *et al.*: Potential role for concurrent abnormalities of the cyclin D1, p16CDKN2 and p15CDKN2B genes in certain B cell non-Hodgkin's lymphomas. Functional studies in a cell line (Granta 519). *Leukemia* 11:64-72, 1997
- Joerg H, Fries HR, Meijerink E, Stranzinger GF: Red coat color in Holstein cattle is associated with a deletion in the MSHR gene. *Mamm Genome* 7:317-318, 1996
- Kamb A, Gruis NA, Weaver-Feldhaus J, *et al.*: A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264:436-440, 1994a
- Kamb A, Shattuck-Eidens D, Eeles R, *et al.*: Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 8:23-26, 1994b
- Kamijo T, Zindy F, Roussel MF, *et al.*: Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 91:649-659, 1997
- Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ: Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc Natl Acad Sci USA* 95:8292-8297, 1998
- Kefford RF, Salmon J, Shaw HM, Donald JA, McCarthy WH: Hereditary melanoma in Australia. Variable association with dysplastic nevi and absence of genetic linkage to chromosome 1p. *Cancer Genet Cytogenet* 51:45-55, 1991
- Koppula SV, Robbins LS, Lu D, Baack E, White CR Jr, Swanson NA, Cone RD: Identification of common polymorphisms in the coding sequence of the human MSH receptor (MC1R) with possible biological effects. *Hum Mutat* 9:30-36, 1997
- Liu L, Lassam NJ, Slingerland JM, Bailey D, Cole D, Jenkins R, Hogg D: Germline p16INK4A mutation and protein dysfunction in a family with inherited melanoma. *Oncogene* 11:405-412, 1995
- Liu Q, Neuhause S, McClure M, *et al.*: CDKN2 (MTS1) tumor suppressor gene mutations in human tumor cell lines. *Oncogene* 10:1061-1067, 1995
- Liu L, Goldstein AM, Tucker MA, Brill H, Gruis NA, Hogg D, Lassam NJ: Affected members of melanoma-prone families with linkage to 9p21 but lacking mutations in CDKN2A do not harbor mutations in the coding regions of either CDKN2B or p19ARF. *Genes Chromosomes Cancer* 19:52-54, 1997
- MacGeoch C, Bishop JA, Bataille V, *et al.*: Genetic heterogeneity in familial malignant melanoma. *Hum Mol Genet* 3:2195-2200, 1994
- Mountjoy KG, Robbins LS, Mortrud MT, Cone RD: The cloning of a family of genes that encode the melanocortin receptors. *Science* 257:1248-1251, 1992
- Nancarrow DJ, Palmer JM, Walters MK, *et al.*: Exclusion of the familial melanoma locus (MLM) from the PND/DIS47 and MYCL1 regions of chromosome arm 1p in 7 Australian pedigrees. *Genomics* 12:18-25, 1992
- Nancarrow DJ, Mann GJ, Holland EA, *et al.*: Confirmation of chromosome 9p linkage in familial melanoma. *Am J Hum Genet* 53:936-942, 1993
- Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA: Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature* 368:753-756, 1994
- Norris W: A case of fungoid disease. *Edinb Med Surg J* 16:562-565, 1820
- Pollock PM, Spurr N, Bishop T, *et al.*: Haplotype analysis of two recurrent CDKN2A mutations in 10 melanoma families: evidence for common founders and independent mutations. *Hum Mutat* 11:424-431, 1998
- Quelle DE, Cheng M, Ashmun RA, Sherr CJ: Cancer-associated mutations at the INK4a locus cancel cell cycle arrest by p16INK4a but not by the alternative reading frame protein p19ARF. *Proc Natl Acad Sci USA* 94:669-673, 1997

- Rizos H, Becker TM, Holland EA, Kefford RF, Mann GJ: Differential expression of p16INK4a and p16beta transcripts in B- lymphoblastoid cells from members of hereditary melanoma families without CDKN2A exon mutations. *Oncogene* 15:515-523, 1997
- Robbins LS, Nadeau JH, Johnson KR, et al: Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* 72:827-834, 1993
- Serrano M, Hannon GJ, Beach D: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366:704-707, 1993
- Smith R, Healy E, Siddiqui S, et al: Melanocortin 1 receptor variants in an Irish population. *J Invest Dermatol* 111:119-122, 1998
- Soufir N, Avril MF, Chompret 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* 7:209-216, 1998
- Stadler WM, Olopade OI: The 9p21 region in bladder cancer cell lines: large homozygous deletion inactivate the CDKN2, CDKN2B and MTAP genes. *Urol Res* 24:239-244, 1996
- Stone S, Dayananth P, Jiang P, Weaver-Feldhaus JM, Tavtigian SV, Cannon-Albright L, Kamb A: Genomic structure, expression and mutational analysis of the P15 (MTS2) gene. *Oncogene* 11:987-991, 1995
- Stott FJ, Bates S, James MC, et al: The alternative product from the human CDKN2A locus, p14 (ARF), participates in a regulatory feedback loop with p53 and MDM2. *Embo J* 17:5001-5014, 1998
- Suzuki H, Zhou X, Yin J, et al: Intragenic mutations of CDKN2B and CDKN2A in primary human esophageal cancers. *Hum Mol Genet* 4:1883-1887, 1995
- Vage DI, Lu D, Klungland H, Lien S, Adalsteinsson S, Cone RD: A non-epistatic interaction of agouti and extension in the fox, *Vulpes vulpes*. *Nat Genet* 15:311-315, 1997
- Valverde P, Healy E, Jackson I, Rees JL, Thody AJ: Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet* 11:328-330, 1995
- Valverde P, Healy E, Sikkink S, et al: The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. *Hum Mol Genet* 5:1663-1666, 1996
- Walker GJ, Flores JF, Glendening JM, Lin AH, Markl ID, Fountain JW: Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. *Genes Chromosomes Cancer* 22:157-163, 1998
- Weaver-Feldhaus J, Gruis NA, Neuhausen S, Le Paslier D, Stockert E, Skolnick MH, Kamb A: Localization of a putative tumor suppressor gene by using homozygous deletions in melanomas. *Proc Natl Acad Sci USA* 91:7563-7567, 1994
- Xu X: Val92Met variant of the melanocyte stimulating hormone receptor gene. *Nat Genet* 14:384, 1996
- Zuo L, Weger J, Yang Q, et al: Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet* 12:97-99, 1996