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A comparison of *CDKN2A* mutation detection within the Melanoma Genetics Consortium (GenoMEL)

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ABSTRACT

CDKN2A is the major melanoma susceptibility gene so far identified, but only 40% of three or more case families have identified mutations. A comparison of mutation detection rates was carried out by "blind" exchange of samples across GenoMEL, the Melanoma Genetics Consortium, to establish the false negative detection rates. Denaturing high performance liquid chromatography (DHPLC) screening results from 451 samples were compared to screening data from nine research groups in which the initial mutation screen had been done predominantly by sequencing. Three samples with mutations identified at the local centres were not detected by the DHPLC screen. No additional mutations were detected by DHPLC. Mutation detection across groups within GenoMEL is carried out to a consistently high standard. The relatively low rate of *CDKN2A* mutation detection is not due to failure to detect mutations and implies the existence of other high penetrance melanoma susceptibility genes.

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1. Introduction

GenoMEL (the Melanoma Genetics Consortium; http://www.genomel.org) is comprised of groups on four continents working on high penetrance genes in multiple case melanoma families. The major susceptibility locus for melanoma is *CDKN2A* on chromosome 9p. The majority of causal mutations at this locus, many of which are single base pair substitutions in exons 1α and 2, affect the function of the protein p16INK4a. Some of the mutations in exon 2 also impact on the alternative splice product of the locus, p14ARF. Since p16INK4a was first described as a melanoma susceptibility gene,[1–3] increasing numbers of mutations at the locus have been described.

Less common types of germline mutation have been reported, including a promoter variant that creates an alternative initiation codon[4,5] and a deep intronic mutation common in England.[6] A comprehensive screen of the intronic regions of *CDKN2A* identified two additional putative intronic mutations. However, in English pedigrees at least, these do not appear to explain predisposition to melanoma in a significant proportion of families.[7]

Recently, rare causal mutations have been identified in exon 1β; these mutations impact p14ARF alone. Specifically, a germline deletion not affecting p16INK4a was reported in 2001,[8] a 16 base pair insertion in exon 1β was detected in a Spanish melanoma family,[9] and a number of pedigrees with exon 1β splice site variants have been described.[10,11] Finally, a recent screen of 146 English melanoma families identified a small number of pedigrees with germline deletions at the 9p21 locus.[12]

Within GenoMEL, the overall proportion of families with identifiable mutations is relatively low and there is considerable variation between centres.[13,14] In a study from Italy, 33% of pedigrees with two or more cases of melanoma had mutations,[15] whereas a Spanish study showed that 17% of melanoma families had *CDKN2A* mutations.[16] In Australia, lower percentages have been reported, e.g. 8.4% of two or more case families.[17] The variation between centres may result from the founder effects and the variable presence of other as yet unidentified susceptibility genes such as the putative gene at 1p22.[18] There may also be an effect of the environment. Clustering in families in areas of high sun exposure such as Australia may result from enhanced contribution of lower penetrance susceptibility genes such as MC1R. Indeed, compared to Europe, there is almost a doubling of the penetrance of *CDKN2A* mutations in Australia which is thought to be due to a higher ultraviolet radiation flux.[19]

Another possibility, however, is that groups had failed to identify significant numbers of mutations at the *CDKN2A* locus, particularly since early mutation detection studies often used the single-stranded conformational polymorphism (SSCP) analysis rather than sequencing. GenoMEL, therefore, designed an audit to evaluate the overall quality of mutation detection across the entire *CDKN2A* locus. We also investigated the utility of denaturing high performance liquid chromatography (DHPLC) as a screening approach to be used by GenoMEL in large numbers of samples. Samples that had initially been genotyped at the centre of origin by sequencing (eight centres) or by SSCP (one centre) were sent to Leeds, UK, for screening with DHPLC. The study also therefore provides a comparison of sequencing with DHPLC.

2. Materials and methods

2.1. Samples and general organisation

The core groups within GenoMEL agreed to send samples to the Division of Epidemiology and Biostatistics of the Cancer Research UK Clinical Centre at Leeds, UK. The participating groups were from Barcelona, Spain (BCN), Leiden University Medical Center, the Netherlands (LUMC), Queensland Institute of Medical Research, Australia (QIMR). Massachusetts General Hospital, Boston, USA (MGH), the National Cancer Institute, Washington, USA (NCI/USA), an NCI group collaborating with Emilia-Romagna, Italy (NCI/Italy), the University of Genoa, Italy (U Genoa), the University of Pennsylvania, Philadelphia, USA (U Penn) and Westmead Institute for Cancer Research, New South Wales, Australia (WICR). The samples were labelled by the study number alone, and therefore the Leeds group was blind to the mutation status of the sample.

All groups provided DNA from two melanoma cases from families with three or more melanoma patients that had been screened by this group, whether a mutation had been detected or not. In each case, the initial mutation detection screen carried out at the centre of origin was by sequencing, with the exception of WICR, where the primary screen was by SSCP for *CDKN2A* exon 1 and by sequencing for exon 2.

The samples were processed by the Leeds group and the results sent to the NCI in Washington Bethesda, MD, where DHPLC audit results were pooled with the original groups' results. Only coding mutations were assessed; polymorphisms were not considered in this analysis.

2.2. Statistical analysis

The DHPLC results from Leeds were compared to the results from the original centres using two units of evaluation: "sample" and "exon". Sample summarized the results over the five different exons evaluated. Exon separately examined *CDKN2A* exons 1α, 1β, 2 and 3 and *CDK4* exon 2. Two measures of evaluation were used: failure and discrepancy. Failure was defined as the percentage of samples or exons that failed the DHPLC assay. Discrepancy was the proportion of inconsistencies between DHPLC and the original centre's results. To confirm discrepancies and eliminate any sample handling errors at any point in the process, all samples with initial evidence for discrepant results were sequenced at the University of Toronto (D. Hogg).

2.3. PCR amplification

The four exons of *CDKN2A* (exons 1α, 1β, 2 and 3) and *CDK4* exon 2 were amplified from genomic DNA by PCR, using previously described primers (Table 1).[2,3,20] PCR was carried out in a total volume of 25 μl, using 25 ng genomic DNA, 0.2 mM dNTPs, 50 μM each primer, 5% (v/v) DMSO, 1.5 mM $MgCl_2$ and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Warrington, UK), in the reaction buffer supplied by the manufacturer. PCR amplification conditions were as fol-