Duplication of CXC chemokine genes on chromosome 4q13 in a melanoma-prone family

Xiaohong R. Yang[1,*], **Kevin Brown**[1], **Maria T. Landi**[1], **Paola Ghiorzo**[2], **Celia Badenas**[3], **Mai Xu**[1], **Nicholas K. Hayward**[4], **Donato Calista**[5], **Giorgio Landi**[5], **William Bruno**[2], **Giovanna Bianchi-Scarrà**[2,6], **Paula Aguilera**[7], **Susana Puig**[7,8], **Alisa M. Goldstein**[1], and **Margaret A. Tucker**[1]

[1]Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, DHHS, MD, US

[2]DOBIG, Department of Oncology, Biology and Genetics, University of Genoa, Genoa, Italy

[3]Biochemistry and Molecular Genetics Department, Hospital Clinic of Barcelona, IDIBAPS, Barcelona, Spain

[4]Oncogenomics Laboratory, Queensland Institute of Medical Research, Brisbane, QLD, Australia

[5]Maurizio Bufalini Hospital, Cesena, Italy

[6]Laboratory of Genetics of Rare Hereditary Cancers, San Martino Hospital, Genoa, Italy

[7]Melanoma Unit, Dermatology Department, Hospital Clinic of Barcelona, IDIBAPS, Barcelona, Spain

[8]CIBER de Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Spain

Summary

Copy number variations (CNVs) have been shown to contribute substantially to disease susceptibility in several inherited diseases including cancer. We conducted a genome-wide search for CNVs in blood-derived DNA from 79 individuals (62 melanoma patients and 17 spouse controls) of 30 high-risk melanoma-prone families without known segregating mutations using genome-wide comparative genomic hybridization (CGH) tiling arrays. We identified a duplicated region on chromosome 4q13 in germline DNA of all melanoma patients in a melanoma-prone family with three affected siblings. We confirmed the duplication using quantitative PCR and a custom-made CGH array design spanning the 4q13 region. The duplicated region contains 10 genes, most of which encode CXC chemokines. Among them, *CXCL1* (melanoma growth-stimulating activity α) and *IL8* (interleukin 8) have been shown to stimulate melanoma growth *in vitro* and *in vivo*. Our data suggests that the alteration of CXC chemokine genes may confer susceptibility to melanoma.

Keywords

Familial melanoma; Germline copy number variations; disease susceptibility; CXC chemokines; chromosome 4q13

---

*Xiaohong Rose Yang, Ph.D., M.P.H., Genetic Epidemiology Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute, NIH, DHHS, 6120 Executive Blvd., Rm. 7014, Bethesda, MD 20892-7236. Tel (301)594-7804; Fax (301)402-4489; royang@mail.nih.gov.

Cutaneous malignant melanoma is an etiologically heterogeneous disease with genetic, host, and environmental factors, as well as their interactions contributing to its development (Tucker and Goldstein, 2003). Approximately 10% of melanoma cases occur in a familial setting (Goldstein and Tucker, 2001). To date, two high-risk melanoma susceptibility genes, *CDKN2A* on chromosome 9p21 and *CDK4* on 12q14, have been identified. Germline mutations of these two genes account for only a small proportion of familial melanoma susceptibility, suggesting the existence of other high-risk genes. Recently, copy number variants (CNVs) have been shown to contribute substantially to disease susceptibility in several inherited diseases including cancer (Kuiper et al., 2010). Specifically, a recent genome-wide CNV mapping study reported the identification of a major susceptibility gene for a familial cancer, chordoma (Yang et al., 2009), suggesting that screening for complex genomic rearrangements that co-segregate with disease in families may provide a powerful alternative to traditional gene-mapping approaches.

We conducted a genome-wide search for CNVs in 30 high-risk melanoma-prone families without known segregating mutations using a whole-genome human array–comparative genomic hybridization (array-CGH) chip (Nimblegen 385K; average probe spacing, 7 kb). The families were from the United States and ascertained through health care professionals or self referrals. The families included at least two living first degree relatives with a history of invasive melanoma. All family members who were willing to participate in the study provided written informed consent under an NCI IRB-approved protocol. Each underwent a full-body skin examination and completed risk factor questionnaires for sun-related exposures. All diagnoses of melanoma were confirmed by histologic review of pathologic material or by pathology reports. We analyzed blood-derived genomic DNA from 79 individuals including 62 melanoma patients (1–4 patients per family) and 17 spouses. We used the Nexus Copy Number built-in Rank Segmentation algorithm to identify significant CNVs ($P=1\times10^{-6}$; number of probes per segment 10; log_2 ratio>0.25 for gains and <−0.25 for losses). We focused on CNVs that were not present in the 17 spouses, did not overlap with previously reported CNVs, were located within gene regions, and occurred in all melanoma patients within a family.

We identified a duplicated region located on 4q13 in two melanoma patients (II-1 and II-3) in the family shown in Fig. 1. Three individuals (II-1, II-3, and II-4) had a confirmed history of melanoma at the time of initial examination (Fig. 1). Similar to other American melanoma-prone families, all of the melanoma patients had dysplastic nevi (DN), and relatively early onset of melanoma; one patient had multiple primary melanomas. The siblings' mother (I-2) had confirmed squamous cell carcinomas of the skin and reportedly had lung cancer and melanoma; the maternal grandmother and a maternal cousin also were reported to have melanoma. None of these three melanomas could be confirmed.

To validate the duplication and to examine the co-segregation of the duplication with melanoma status, we developed 2 quantitative PCR (qPCR) assays targeting two genes in this region, *CXCL3* exon 4 and *CXCL6* exon 4, respectively, and analyzed all 7 individuals with DNA available in this family. qPCR analyses confirmed the duplication in all three affected siblings, II-1, II-3, and II-4 (Fig. 2a). The unaffected father (I-1), the unaffected sibling (II-2), and an unaffected grandson (IV-1), did not have the duplication. The unaffected offspring (III-1) of one of the affected patients (II-1) also had the duplication. However, she was only 23 years old at ascertainment, had extensive number of nevi including DN, and had used sun protection for most of her life.

To further confirm the 4q13 duplication and to better define the breakpoints of the amplicons, we analyzed genomic DNA from fifteen individuals (individuals II-1, II-2, II-3, II-4, and III-1 in the family with the 4q13 duplication, 5 melanoma patients from families

without the 4q13 duplication, and 5 unaffected controls) using a Nimblegen custom-made fine-tiling CGH array spanning the 4q13 region (average probe spacing, 15 bp). We confirmed the duplication in all three affected siblings and in individual III-1. As expected, the duplications in these four individuals were identical in size and location. In contrast, no duplication was observed in II-2, or in five other melanoma patients from five families that did not carry the duplication, or in five controls. We subsequently PCR amplified and sequenced the junction fragments from individuals II-1 and III-1 and determined that the duplication was 257kb (74663132 to 74919990 bp, hg19) with a head-to-tail tandem orientation (Fig. 2b, 2c). Bioinformatic analysis revealed that the breakpoints were located at or near repetitive short and long interspersed repeat (SINE and LINE) elements (Fig. 2b). In contrast, no junction fragment was amplified from individual II-2 who did not carry the duplication.

The duplicated region was not present in the other individuals evaluated by array-CGH. In addition, the 4q13 duplication was not observed in 318 control chromosomes (159 control subjects) by qPCR, suggesting that the duplication is unlikely a common polymorphism. Furthermore, the duplication was not observed in index patients from additional *CDKN2A/CDK4* mutation-negative melanoma-prone families, including 16 American, 182 Italian, 170 Spanish, and 96 Australian families using either qPCR or array-CGH.

The duplicated region affected 10 genes, including *IL8* (interleukin 8), *CXCL6* (granulocyte chemotactic protein-2), *PPBPL1* (pro-platelet basic protein-like 1), *PF4V1* (platelet factor-4 variant 1), *CXCL1* (melanoma growth-stimulating activity α), *PF4* (platelet factor-4), *PPBP* (pro-platelet basic protein), *CXCL5* (chemokine (C-X-C motif) ligand 5), *CXCL3* (melanoma growth-stimulating activity γ), and *PPBPL2* (pro-platelet basic protein-like 2). *IL8* and *PPBPL2* were partially affected and the remaining eight genes were completely contained in the duplicated region (Fig. 2b). Most of these genes belong to a family of CXC chemokines, which are characterized by one amino acid between the first and second cysteine residues. Among them, *CXCL1*, *CXCL3*, *CXCL5*, *CXCL6*, *PPBP*, and *IL8* contain a three amino acid ELR motif (glutamine–leucine–arginine; ELR+) between the N-terminus and the first cysteine, and function as potent promoters of angiogenesis, therefore promoting tumorigenesis and metastasis (Payne and Cornelius, 2002).

CXCL1 has been shown to be up-regulated in melanoma cells and play an important role in melanoma pathogenesis (Dhawan and Richmond, 2002), with secretion of *CXCL1* in melanoma cell lines 6- to 16-fold higher than in normal melanocytes (Norgauer et al., 1996). Overexpression of *CXCL1* in melanocytes has been associated with enhanced growth and tumor formation in nude mice (Balentien et al., 1991). Blocking antibodies to either *CXCL1* or its receptor, chemokine (C-X-C motif) receptor 2 (*CXCR2*), inhibited melanoma cell growth and reduced angiogenic activity (Haghnegahdar et al., 2000). Similarly, *IL8* was the first chemokine reported to induce melanoma cell chemotactic and haptotactic migration (Wang et al., 1990). Neutralizing antibodies to the *IL8* receptors *CXCR1* and *CXCR2*, as well as to *IL8* itself, inhibited melanoma cell proliferation and invasive potential (Varney et al., 2003), suggesting the potential for these chemokines and their receptors in melanoma treatment. In a study identifying gene expression signatures in melanoma progression, Haqq et al. found that elevated expression of *CXCL1* not only distinguished primary tumors from moles but also was useful in identifying metastases (Haqq et al., 2005). Similarly, data from molecular profiling analyses also found upregulation of *CXCL3* and *IL8* in melanoma tumors and metastases (Lukk et al., 2010) (Mauerer et al., 2011) (Bertucci et al., 2007). In addition, recent data suggest that ciglitazone, an oral thiazolidinedione anti-diabetic drug, inhibited growth and survival of melanoma cells through repressing the expression and secretion of *CXCL1*, while the recombinant *CXCL1* abrogated the apoptotic effect induced by ciglitazone and inhibition of *CXCL1* production mimicked the effects of ciglitazone on

growth inhibition (Botton et al., 2011). Furthermore, *CXCL1* inhibition by ciglitazone was mediated by the decreased expression of microphthalmia-associated transcription factor (*MITF*), the master gene of melanocyte differentiation. These results further highlight the importance of *CXCL1* in melanoma tumorigenicity and its potential as a therapeutic target.

In summary, we identified a germline duplication that may explain the melanoma susceptibility in a melanoma-prone family. The duplicated region contains genes that are known to play important roles in melanoma development and progression. Although the duplication is very rare, other types of variants such as point mutations and small insertions/deletions in these genes may explain susceptibility in additional melanoma families and therefore evaluations of variations within this novel duplication are needed to further determine the role of these genes in melanoma susceptibility.

Acknowledgments

References

Balentien E, Mufson BE, Shattuck RL, Derynck R, Richmond A. Effects of MGSA/GRO alpha on melanocyte transformation. Oncogene. 1991; 6:1115–24. [PubMed: 1861861]

Bertucci F, Pages C, Finetti P, Rochaix P, Lamant L, Devilard E, Nguyen C, Houlgatte R, Birnbaum D, Xerri L, et al. Gene expression profiling of human melanoma cell lines with distinct metastatic potential identifies new progression markers. Anticancer Res. 2007; 27:3441–9. [PubMed: 17970093]

Botton T, Puissant A, Cheli Y, Tomic T, Giuliano S, Fajas L, Deckert M, Ortonne JP, Bertolotto C, Tartare-Deckert S, et al. Ciglitazone negatively regulates CXCL1 signaling through MITF to suppress melanoma growth. Cell Death Differ. 2011; 18:109–21. [PubMed: 20596077]

Dhawan P, Richmond A. Role of CXCL1 in tumorigenesis of melanoma. J Leukoc Biol. 2002; 72:9–18. [PubMed: 12101257]

Goldstein AM, Tucker MA. Genetic epidemiology of cutaneous melanoma: a global perspective. Arch Dermatol. 2001; 137:1493–6. [PubMed: 11708953]

Haghnegahdar H, Du J, Wang D, Strieter RM, Burdick MD, Nanney LB, Cardwell N, Luan J, Shattuck-Brandt R, Richmond A. The tumorigenic and angiogenic effects of MGSA/GRO proteins inmelanoma. J Leukoc Biol. 2000; 67:53–62. [PubMed: 10647998]

Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodabakhsh D, Pulliam BL, Federman S, Miller JR 3rd, Allen RE, Singer MI, et al. The gene expression signatures of melanoma progression. Proc Natl Acad Sci U S A. 2005; 102:6092–7. [PubMed: 15833814]

Kuiper RP, Ligtenberg MJ, Hoogerbrugge N, Geurts Van Kessel A. Germline copy number variation and cancer risk. Curr Opin Genet Dev. 2010; 20:282–9. [PubMed: 20381334]

Lukk M, Kapushesky M, Nikkila J, Parkinson H, Goncalves A, Huber W, Ukkonen E, Brazma A. A global map of human gene expression. Nat Biotechnol. 2010; 28:322–4. [PubMed: 20379172]

Mauerer A, Roesch A, Hafner C, Stempfl T, Wild P, Meyer S, Landthaler M, Vogt T. Identification of new genes associated with melanoma. Exp Dermatol. 2011; 20:502–7. [PubMed: 21410771]

Norgauer J, Metzner B, Schraufstatter I. Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. J Immunol. 1996; 156:1132–37. [PubMed: 8557989]

Payne AS, Cornelius LA. The role of chemokines in melanoma tumor growth and metastasis. J Invest Dermatol. 2002; 118:915–22. [PubMed: 12060384]

Tucker MA, Goldstein AM. Melanoma etiology: where are we? Oncogene. 2003; 22:3042–52. [PubMed: 12789279]

Varney ML, Li A, Dave BJ, Bucana CD, Johansson SL, Singh RK. Expression of CXCR1 and CXCR2 receptors in malignant melanoma with different metastatic potential and their role in interleukin-8 (CXCL-8)-mediated modulation of metastatic phenotype. Clin Exp Metastasis. 2003; 20:723–31. [PubMed: 14713106]

Wang JM, Taraboletti G, Matsushima K, Van Damme J, Mantovani A. Induction of haptotactic migration of melanoma cells by neutrophil activating protein/interleukin-8. Biochem Biophys Res Commun. 1990; 169:165–70. [PubMed: 2190552]

Yang XHR, Ng D, Alcorta DA, Liebsch NJ, Sheridan E, Li SF, Goldstein AM, Parry DM, Kelley MJ. T (brachyury) gene duplication confers major susceptibility to familial chordoma. Nature Genetics. 2009; 41:1176–1178. [PubMed: 19801981]

Significance

Several CXC chemokines are known to play important roles in melanoma development and progression. Here we show for the first time that CXC chemokine genes may confer major susceptibility to familial melanoma. Variations in these genes, including CNVs and single-nucleotide mutations, should be screened in melanoma-prone families without a defined etiology. Our results further support the role of rare, inherited copy number variations in cancer susceptibility.
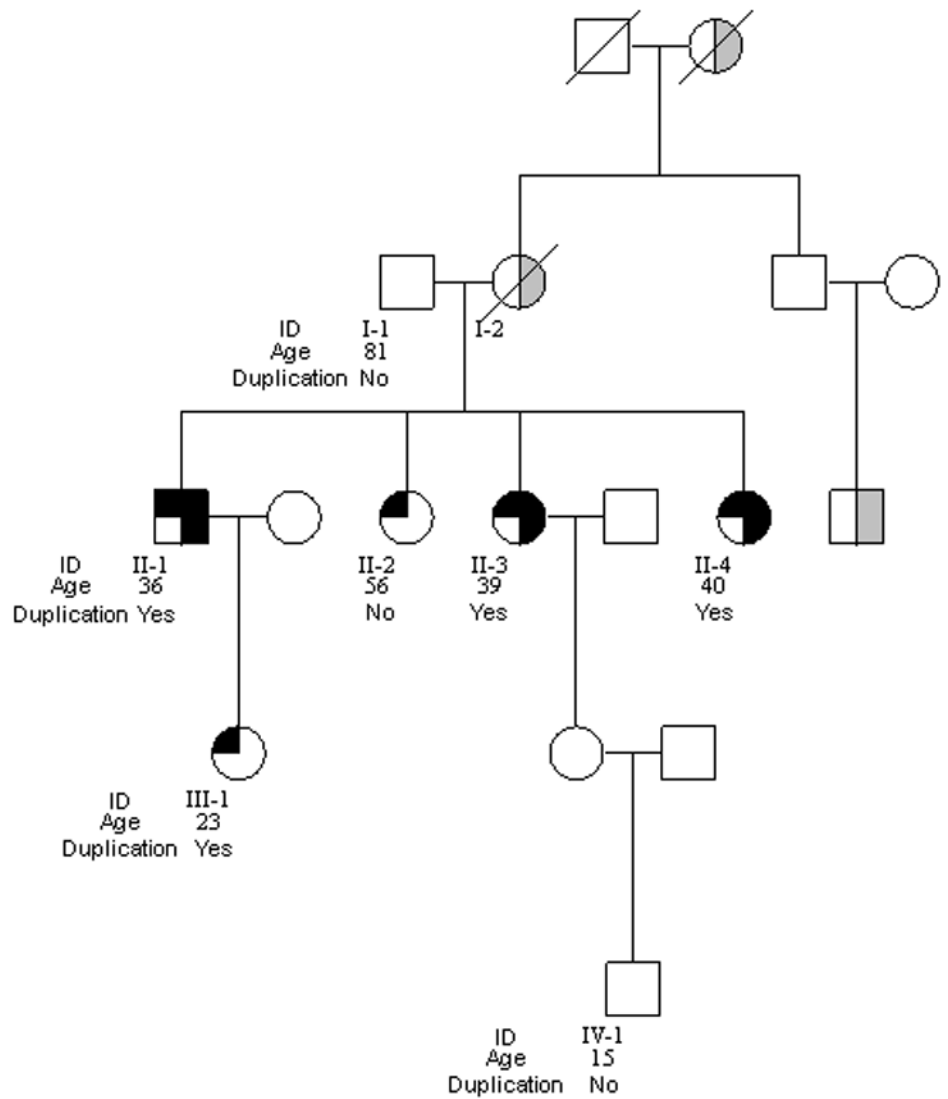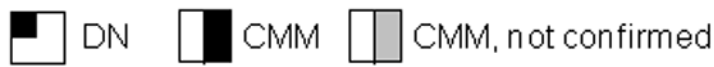
Fig. 1.
The pedigree of the melanoma family with the 4q13 duplication. Age as shown is age at diagnosis for melanoma patients and age at evaluation for unaffected individuals. II-1 and II-3 were analyzed by whole-genome array-CGH; I-1, II-1, II-2, II-3, II-4, III-1, and IV-1 were analyzed by qPCR; II-1, II-2, II-3, II-4, and III-1 were analyzed by custom-made 4q13 tiling arrays.
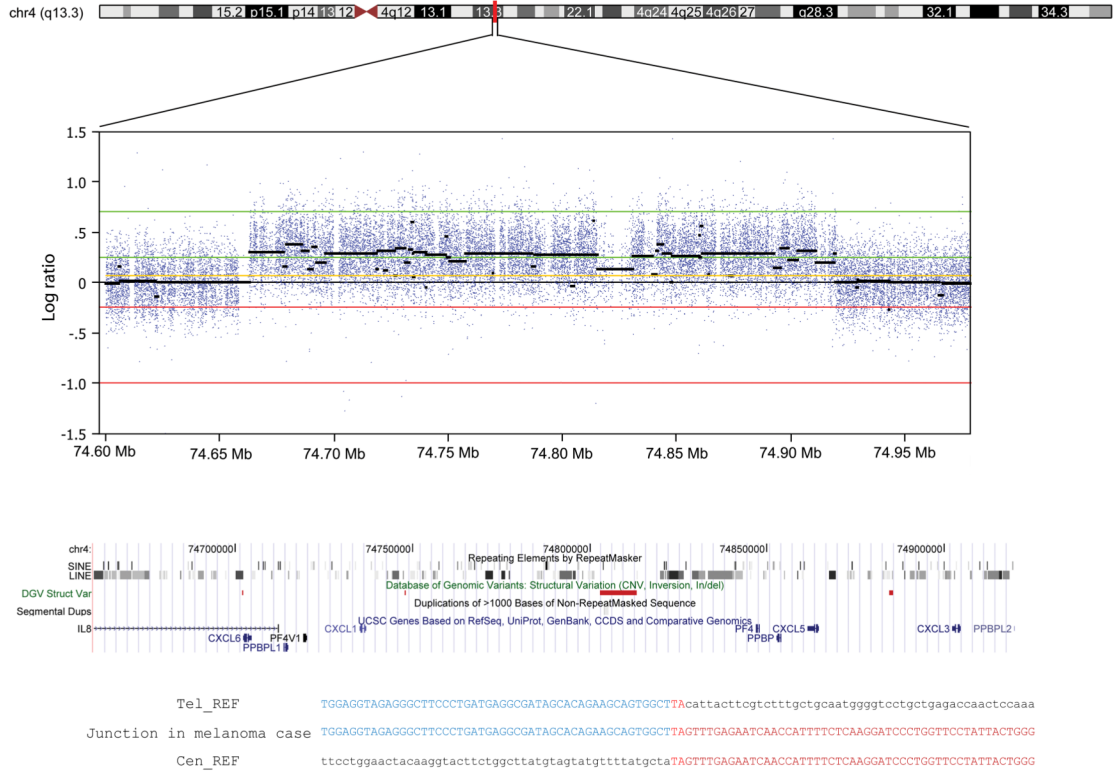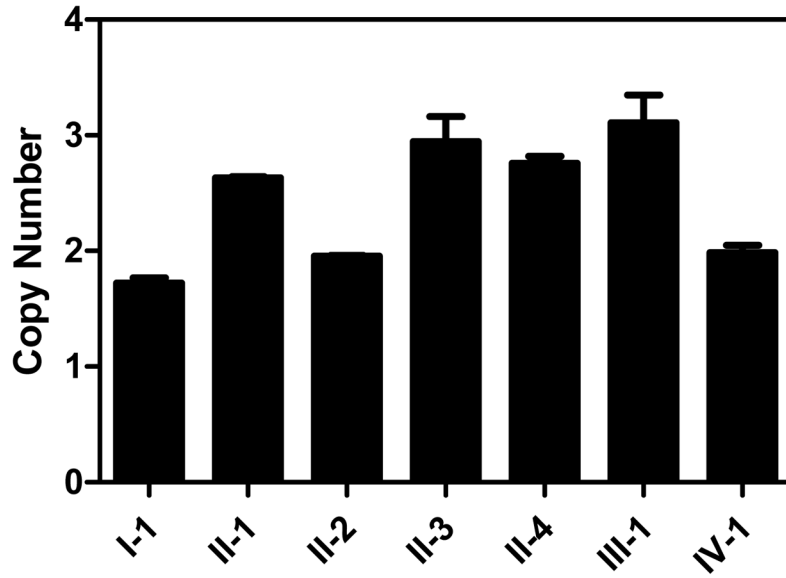
Fig. 2.
The 4q13 duplication identified in the melanoma-prone family. Panel a. Quantitative PCR (qPCR) of *CXCL3* in genomic DNA of the melanoma family. Each qPCR assay was performed in duplicate. Results of qPCR assays for each individual are shown as a point estimate and 1 SD interval expressed as fold-difference compared to a reference sample. Data for *CXCL6* were similar (not shown). Panel b. The duplication in melanoma patient II-3 by 4q13-focused array-CGH. Previously-reported CNVs listed in the Database of Genomic Variations are shown as red bars and none were located within genes. The

duplicated region contains short and long interspersed repeat elements (SINE and LINE). Panel c. Breakpoint junction of the duplication showing a head-to-tail orientation. Tel-REF: telomeric reference sequence; Cen-REF: centromeric reference sequence. Matched sequences between reference and the melanoma patient are shown in colors (blue for telomeric and red for centromeric).