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SHORT REPORT

Does multilocus inherited neoplasia alleles syndrome have severe clinical expression?

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ABSTRACT

Importance Genetic testing of hereditary cancer using comprehensive gene panels can identify patients with more than one pathogenic mutation in high and/or moderate-risk-associated cancer genes. This phenomenon is known as multilocus inherited neoplasia alleles syndrome (MINAS), which has been potentially linked to more severe clinical manifestations.

Objective To determine the prevalence and clinical features of MINAS in a large cohort of adult patients with hereditary cancer homogeneously tested with the same gene panel.

Patients and methods A cohort of 1023 unrelated patients with suspicion of hereditary cancer was screened using a validated panel including up to 135 genes associated with hereditary cancer and phakomatoses.

Results Thirteen (1.37%) patients harbouring two pathogenic mutations in dominant cancer-predisposing genes were identified, representing 5.7% (13/226) of patients with pathogenic mutations. Most (10/13) of these cases presented clinical manifestations associated with only one of the mutations identified. One case showed mutations in *MEN1* and *MLH1* and developed tumours associated with both cancer syndromes. Interestingly, three of the double mutants had a young age of onset or severe breast cancer phenotype and carried mutations in moderate to low-risk DNA damage repair-associated genes; two of them presented biallelic inactivation of *CHEK2*. We included these two patients for the sake of their clinical interest although we are aware that they do not exactly fulfil the definition of MINAS since both mutations are in the same gene.

Conclusions and relevance Genetic analysis of a broad cancer gene panel identified the largest series of patients with MINAS described in a single study. Overall, our data do not support the existence of more severe manifestations in double mutants at the time of diagnosis although they do confirm previous evidence of severe phenotype in biallelic *CHEK2* and other DNA repair cancer-predisposing genes.

INTRODUCTION

Hereditary cancer syndromes account for 5%–10% of all patients with cancer.¹ Patients with these syndromes are carriers of pathogenic mutations in high or moderate-penetrance genes and are at

risk of developing cancer at an early age as well as multiple synchronous or metachronous tumours. It is important to identify these patients because they will require specialised, long-term care and both they and their families can benefit from clinical follow-up appropriate to their risk, together with proper reproductive choices. One of the challenges in genetic counselling of these disorders is dealing with clinical heterogeneity and overlapping clinical manifestations. The phenotype variability could be explained by many factors, in isolation or in combination, such as incomplete penetrance, allelic and genetic heterogeneity, existence of genetic modifiers, environmental factors and stochastic events.^{2,3} Genetic diagnosis of these conditions has evolved over the last decade thanks to the introduction of next-generation sequencing, a cost-effective solution in terms of cost and time for the simultaneous sequencing of multiple genes. These new approaches for sequencing have led to the development of gene panels that contain clearly defined high-penetrance genes and moderate or even low-penetrance genes, arbitrary defined with a relative risk below 4 (moderate) or 2 (low). The use of these genes in the clinical setting is a matter of discussion and some clinical geneticists and genetic counsellors are reluctant to screen them for clinical purposes due to the uncertainty of changing medical management in carriers and in non-carriers of mutations in these genes because clinical utility has not yet been clearly established.⁴ Moreover, the use of large gene panels can lead to unexpected and complex findings, for example, to identify patients with more than one pathogenic mutation in genes implicated in different cancer syndromes.^{5–7} The term ‘MINAS’ (multilocus inherited neoplasia alleles syndrome) was coined by Whitworth and colleagues⁸ in a *JAMA Oncology* review in which the authors presented their experience (five cases) and the literature review (82 cases) of patients with cancer with two pathogenic mutations in hereditary cancer genes. No clear conclusion was reached and a database was created to record such cases (<http://databases.lovd.nl/shared/diseases/04296>); as of July 2018 it contained only 40 entries, all but one from the Whitworth group. Very recently, the same group presented data from a series of 460 patients with two or more tumours identifying two additional cases of MINAS.⁹



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Table 1 Description of patients with MINAS identified

| ID | Phenotype | Tumour/condition | Age Dx | Current age (β)/death (E) | Mutation A* | Mutation B* |
|-----------------|------------------------|---|----------------------|---------------------------|---|--|
| Fam-1 | AFAP | Colorectal polyposis | 76 | 77 (E) | <i>APC</i> [c.423-3T>A; p.(Arg141Serfs*8)] | <i>BRCA1</i> [c.1961delA; p.(Lys654Serfs*47)] |
| Fam-2 (proband) | MEN1 | Neuroendocrine tumour Pituitary adenoma Parathyroid Hyperplasia Hepatic haemangiomas | 41 36 39 29 | 45 (β) | <i>MLH1</i> [c.244A>G; p.(Thr82Ala)] | <i>MEN1</i> (c.784-9G>A; p.[Lys267Valfs*28,Arg280Serfs*2]) |
| Fam-2 (sister) | HNPCC, MEN1 | Uterine carcinoma Colorectal cancer Haemangiomas Hyperparathyroidism | 41 48 45 45 | 52 (β) | <i>MLH1</i> [c.244A>G; p.(Thr82Ala)] | <i>MEN1</i> (c.784-9G>A; p.[Lys267Valfs*28,Arg280Serfs*2]) |
| Fam-3 | HBOC | Ovarian cancer | 45 | 51 (β) | <i>BRCA1</i> [c.607C>T; p.(Arg1203*)] | <i>TP53</i> [c.659A>G; p.(Tyr220Cys)] |
| Fam-4 | Tuberous sclerosis | Subcutaneous benign tumours Epilepsy | 6 | 28 (β) | <i>TSC2</i> [c.5227C>T; p.(Arg1743Trp)] | <i>RAD51D</i> [c.694C>T; p.(Arg232*)] |
| Fam-5 | Reed's syndrome | Cutaneous leiomyomas | 40 | 47 (β) | <i>FH</i> (c.905-2A>G; p.?) | <i>BARD1</i> [c.157delT; p.(Cys53Valfs*5)] |
| Fam-6 | AFAP | Colorectal polyposis Colorectal cancer | 52 53 | 55 (β) | <i>APC</i> [c.5826_5829del; p.(Asp1942Glu fs*27)] | <i>EXO1</i> [c.1900C>T; p.(Arg634*)] |
| Fam-7 (proband) | HBOC | Ovarian cancer | 51 | 52 (β) | <i>BRCA1</i> [c.2309C>A; p.(Ser770*)] | <i>XPA</i> [c.553C>T; p.(Gln185*)] |
| Fam-7 (sister) | HBOC | Ovarian cancer | 37 | 38 (β) | <i>BRCA1</i> [c.2309C>A; p.(Ser770*)] | <i>XPA</i> [c.553C>T; p.(Gln185*)] |
| Fam-8 | HBOC Birt-Hogg-Dubé | Ovarian cancer Pneumothorax (x5) | 66 33 | 68 (β) | <i>FLCN</i> [c.346C>T; p.(Gln116*)] | <i>ERCC3</i> [c.325C>T; p.(Arg109*)] |
| Fam-9 | HBOC | Breast cancer Pancreatic cancer | 54 59 | 59 (β) | <i>PALB2</i> [c.3256 C>T; p.(Arg1086*)] | <i>ATM</i> (c.3802delG; p.Val1268*) |
| Fam-10† | HBOC | Bilateral breast cancer | 35 | 37 (β) | <i>CHEK2</i> (c.433C>T; p.(Arg145Trp)) | <i>CHEK2</i> [c.470T>C; p.(Ile157Thr)] |
| Fam-11† | HBOC | Breast cancer | 42 | 42 (E) | <i>CHEK2</i> (whole gene deletion) | <i>CHEK2</i> [c.499G>A; p.(Gly167Arg)] |
| Fam-12 | HBOC | Breast cancer | 35 | 38 (β) | <i>ATM</i> [c.3712_3716del; p.(Leu1238Lysfs*6)] | <i>FANCA</i> (c.2602-1G>C; p.?) |
| Fam-13 | HBOC | Ovarian cancer | 49 | 70 (β) | <i>SDHB</i> [c.505C>T; p.(Gln169*)] | <i>FANCA</i> [c.3558dupG; p.(Arg1187Glu fs*28)] |

*Cell shadow code: dark grey: high-risk genes, light grey: moderate to low-risk genes (see online supplementary table 1).

†These patients are compound heterozygous for mutations at the same time, therefore they do not strictly fulfil the MINAS first definition.

AFAP, attenuated familial adenomatous polyposis; HBOC, hereditary breast-ovarian cancer; HNPCC, hereditary non-polyposis colorectal cancer; MEN1, multiple endocrine neoplasia type 1; MINAS, multilocus inherited neoplasia alleles syndrome.

The authors highlighted that data gathered in the literature presented inherent ascertainment bias in relation to the genes and patients analysed. Most of the cases studied in the prepanel era were patients with breast and ovarian or colorectal cancer, in which only a few suspected genes were analysed, hence most examples with double mutations are patients with two germline *BRCA1* and *BRCA2* mutations or with constitutional mismatch repair deficiency syndrome, the latter with a clearly defined severe phenotype.^{10–13} In addition, homozygosity for the founder c.1100delC *CHEK2* mutation has been associated with high breast cancer risk relative to heterozygous carriers.¹⁴

We report the genotype and phenotype of the largest unbiased MINAS cohort of patients with hereditary cancer analysed with a comprehensive gene panel that includes almost all hereditary cancer genes described in the literature.

MATERIALS AND METHODS

Our study population is a cohort of 1023 unrelated adult patients with clinical suspicion of hereditary cancer, visited in the Genetic Counselling Unit of the Catalan Institute of Oncology. Institutional review board approval was obtained for panel testing. For genetic testing, we used our validated custom I2HCP gene panel (containing 122–135 genes, depending on the version used).¹⁵ Variant classification was conducted under American College of Medical Genetics and Genomics guidelines.¹⁶ When possible, cosegregation analysis was performed. A brief description of the whole cohort is depicted in online

supplementary table 1 and online supplementary figure 1 (comprehensive analysis is under publication, Feliubadaló *et al*, submitted manuscript). From our cohort of 1023 patients, 16% had multiple tumours. Of them, four patients are part of our MINAS series. For the purposes of this study, only patients with more than one pathogenic/likely pathogenic mutation (hereafter, ‘mutation’) in dominant cancer-predisposing genes are presented (online supplementary table 2). All the mutations reported here were confirmed by Sanger sequencing. All patients underwent a tiered-binned informed consent process. Twenty-four of our 135 panel genes were denominated CORE genes (Feliubadaló *et al*, submitted manuscript). Pathogenic variants in all these genes were returned to patients—including those with an unrelated phenotype, unless patients did not consent for the CORE panel analysis. The remaining genes of the panel were considered as research genes and are used for research purposes and the results are then explained to families in this scenario where cosegregation analysis is requested.

RESULTS

We identified 13 unrelated patients carrying more than one mutation (1.37%) (table 1, figures 1 and 2), representing 5.75% of patients with pathogenic mutations in our study population (the overall mutation detection yield is 22.14%). The referral criteria for each are summarised in online supplementary table 1.

Nine patients had one high-risk gene mutation (Fam-1 to Fam-9); all showed an association with the proband’s clinical

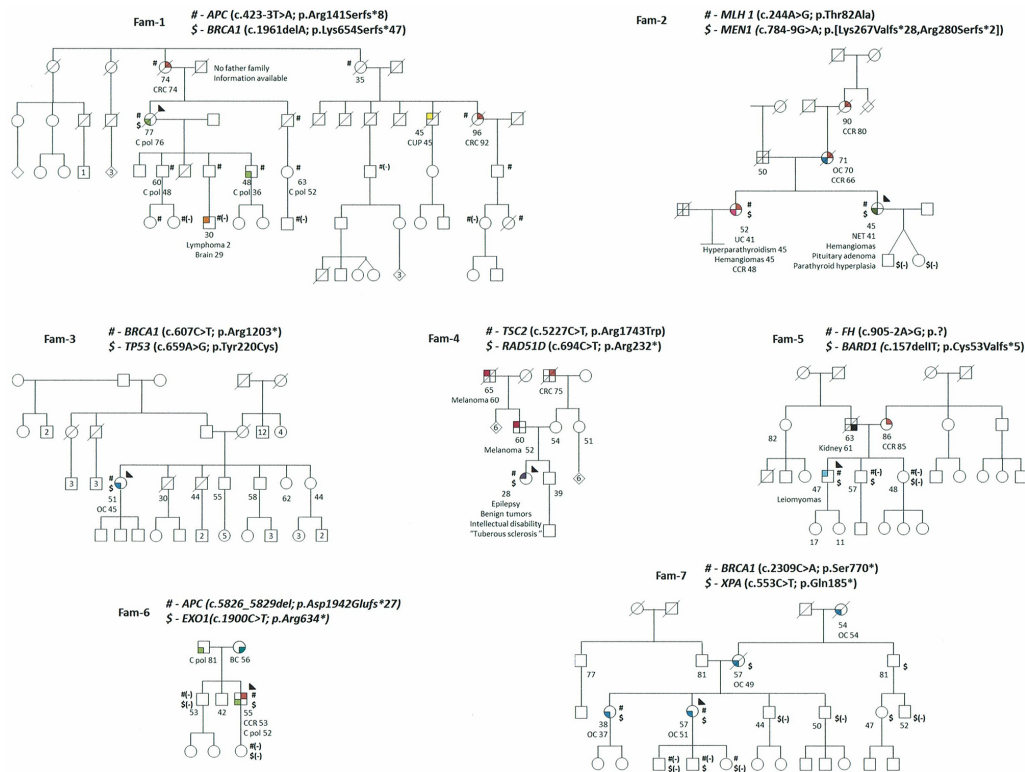


Figure 1 Pedigrees of MINAS patients. Filled quarters of symbols indicate affected patients (each color denotes a specific type of tumor). Current age, age at death and age at diagnosis, when available, are also detailed. Proband is marked by an arrow, carrier status was studied in available relatives, and those carrying the variant are shown with the variant symbol (#,\$) and if genotyped and not carriers a (-) is under the mutation symbol. A number inside a symbol denotes the number of siblings condensed in the symbol. Brain C (light orange), BC: breast cancer (emerald), C pol: colon polyposis (light green), CRC: colorectal cancer (red), CUP: carcinoma of unknown primary (yellow), Kidney Cancer (black), Leiomyomas (light blue), Lymphoma (orange), Melanoma (brown), NET: neuroendocrine tumor (dark green), OC: ovarian cancer (blue), Tuberous sclerosis (purple), UC: uterine carcinoma (pink).

phenotype. The second mutation in these families did not translate into recognised clinical manifestations, except for Fam-2, in which two sisters carry two high-risk mutations; one presented a phenotype consistent with the clinical features of both cancer syndromes (*MLH1* and *MEN1*), whereas the other showed only *MEN1* clinical traits. In Fam-7, two sisters with double mutations (*BRCA1* and *XPA*) presented ovarian cancer at ages 37 and 51, respectively. It should be noted that in two additional patients the second mutation was in a high-risk gene (*BRCA1* and *TP53*), whereas in the remainder the mutations were in genes associated with moderate or low risk of breast, ovarian or colorectal cancer (*RAD51D*, *XPA*, *BARD1*, *EXO1*, *ATM* and *ERCC3*).

Three cases (Fam-10 to Fam-12) with clinical suspicion of hereditary breast-ovarian cancer harbour two mutations in moderate to low breast cancer risk genes: one patient with mutations in *ATM* and *FANCA* genes and two patients with biallelic mutations in *CHEK2*. Two of these patients were diagnosed with young-onset breast cancer (35 years) and the third was diagnosed with metastatic breast cancer at 42 years and had a poor outcome; none had a family history of breast cancer.

The last patient was diagnosed with ovarian cancer before the age of 50 and presented mutations in *SDHB* and *FANCA*, which are difficult to associate with the observed phenotype (Fam-13).

DISCUSSION

The term MINAS was introduced with a view to discerning whether carriers of pathogenic mutations in more than one dominant hereditary cancer gene have specific clinical characteristics or are associated with a more severe phenotype. In our series, 15

patients from 13 families are carriers of two pathogenic mutations in dominant hereditary cancer genes. The most common situation was the presence of a mutation in a high-risk gene associated with the proband's cancer phenotype and a second mutation without current clinical manifestations in the proband or the family. A mixed clinical presentation was only observed in one family where one of carriers of *MEN1* and *MLH1* mutations presented clinical traits of the two hereditary cancer conditions. Interestingly, in three cases of early-onset breast cancer the proband carried two pathogenic mutations in moderate to low-risk genes, suggesting an additive effect of these two mutations. This hypothesis merits further exploration and is additionally supported by Dutch population data for the analysis of the founder c.1100delC *CHEK2* mutation.¹⁴ It is important to note that the two patients who were compound heterozygotes for *CHEK2* mutations do not fulfil the strict definition of MINAS made formerly since both mutations are in the same gene, but we really believe that the fact of observing a severe phenotype in the three instances with mutations in moderate to low cancer risk genes makes it worth highlighting as well as being documented together in the MINAS open database. Notably, we highlight the identification of mutations in known high-risk cancer-associated genes (such as *BRCA1*, *TP53* or *RAD51C*) that, in this context of double mutations, behave as low-penetrance pathogenic variants with no personal or family cancer history. There are different possible reasons for this, such as young age of the proband, incomplete penetrance, a de novo mutation in the proband, genetic mosaicism, lower risk than expected for the specific mutation identified or incomplete/missing family information.

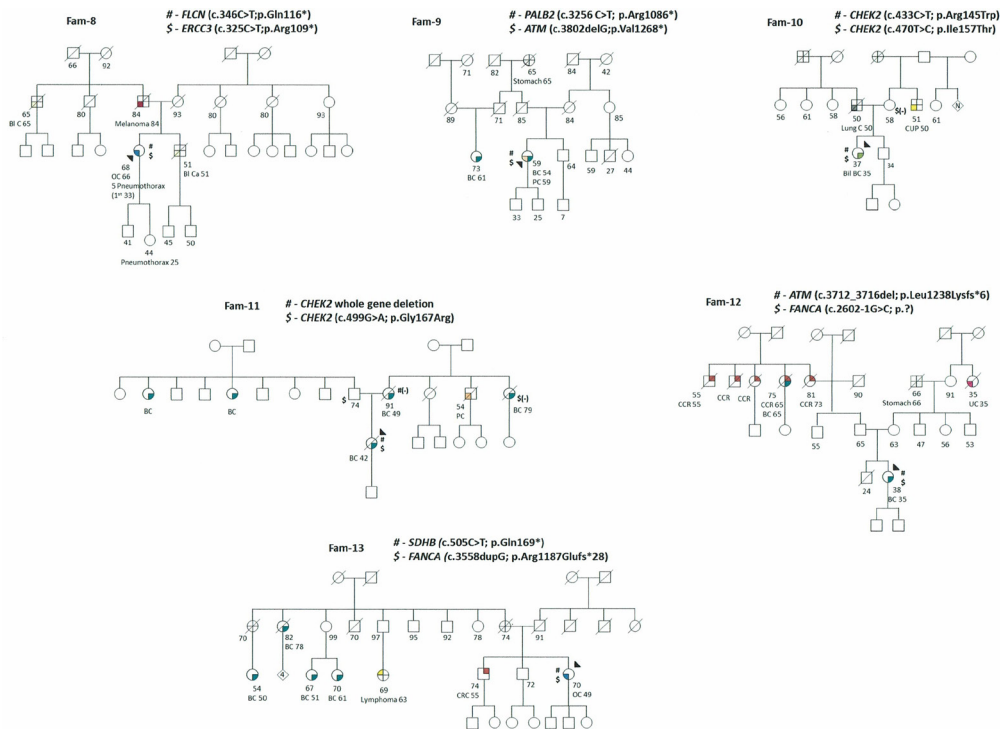


Figure 2 Pedigrees of MINAS patients. Filled quarters of symbols indicate affected patients (each color denotes a specific type of tumor). Current age, age at death and age at diagnosis, when available, are also detailed. Proband is marked by an arrow, carrier status was studied in available relatives, and those carrying the variant are shown with the variant symbol (#,\$) and if genotyped and not carriers a (-) is under the mutation symbol. A number inside a symbol denotes the number of siblings condensed in the symbol. Bl C: bladder cancer (light yellow), BC: breast cancer (emerald), Bil BC: bilateral breast cancer (emerald), CRC: colorectal cancer (red), CUP: carcinoma of unknown primary (yellow), Kidney Cancer (black), LC: lung cancer (grey), Lymphoma (orange), Melanoma (brown), OC: ovarian cancer (blue), PC: pancreas cancer (light orange), Stomach cancer (light grey), Tuberosus sclerosis (purple), UC: uterine carcinoma (pink).

Hopefully, these can be clarified with cosegregation data, functional analysis or tumour profiling. In such situations, genetic counselling, clinical surveillance and cascade testing should be offered since these mutations are in genes of clearly known clinical utility.

In conclusion, further analysis and prospective follow-up of these patients is needed to improve our knowledge of the clinical relevance and consequences of MINAS. Of potential clinical and scientific interest is the putative relation of double mutations in moderate to low cancer risk genes with a severe clinical phenotype in early onset of cancer. As suggested by Whitworth, sharing genetic and clinical data and the continuous clinical update of these patients is crucial. To this end, all our cases have been submitted to the open database created by Whitworth (<https://databases.lovd.nl/shared/diseases/04296>).

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