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Clinical and psychological implications of secondary and incidental findings in cancer susceptibility genes after exome sequencing in patients with rare disorders

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Clinical and psychological implications of secondary and incidental findings in cancer susceptibility genes after exome sequencing in patients with rare disorders

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Abstract

Background/Objectives

Exome sequencing may identify pathogenic variants unrelated with the purpose of the analysis. We investigated the frequency of secondary and incidental findings (SF/IF) in cancer susceptibility genes (CSG), their clinical actionability, and the psychological impact in individuals with an SF/IF (cases) compared with individuals tested due to their cancer history (controls).

Methods

533 exomes ordered for non-cancer conditions. Medical records were reviewed for clinical actionability of SF/IF. Psychological impact was analyzed with the MICRA scale and compared between cases and controls with a propensity score weighting method.

Results

The frequency of SF/IF in CSG was 2.1% (95% CI 1.1% - 3.8%): three *BRCA2*, three *PMS2*, two *SDHB*, and one each in *BRCA1*, *MLH1*, and *RAD51C*. Among relatives, 18 were carriers. Twenty enrolled for surveillance, and a neoplasm was diagnosed in 20%: three paragangliomas and one breast cancer. Cases presented higher MICRA mean scores than controls (21.3 versus 16.2 in MICRA total score, 6.3 versus 4.2 in distress subscale, and 8.3 versus 6.6 in uncertainty subscale; all $p < 0.001$).

Conclusion

SF/IF in CSG were identified in 2.1% of patients. Despite a numerically higher psychological impact, the identification of SF/IF allowed early detection and cancer prevention in families without cancer history.

Key Words: genetic testing, genetics, medical, disease management, genetic predisposition to disease, genetic counseling-

SUMMARY BOX

What is already known on this topic – *summarise the state of scientific knowledge on this subject before you did your study and why this study needed to be done*

- The impact of disclosing a secondary/incidental finding in a cancer susceptibility gene after genomic analyses seems to be low. Nevertheless, the impact of these findings in families of pediatric patients undergoing genomic analysis for non-oncological conditions is still largely unknown

What this study adds – *summarise what we now know as a result of this study that we did not know before*

- This is the first case control study comparing the psychological impact of secondary/incidental findings in cancer susceptibility genes. We demonstrate a high clinical actionability for cancer surveillance in carriers, despite a numerically higher psychological impact compared to carriers identified due to their family history of cancer.

How this study might affect research, practice or policy – *summarise the implications of this study*

- It provides evidence to support the need of genetic counseling to help coping with secondary/incidental findings and it warrants further research on new genetic delivery models to lessen the psychological impact of secondary findings.

Words 2996; 3 figures; 4 Supplementary tables

INTRODUCTION

Exome sequencing is a next generation sequencing tool currently used in patients with suspected rare genetic conditions. In individuals with a clinical history of developmental delay or intellectual disability exome sequencing has become a routine testing practice and it is also used as one of the first-tier diagnostic test in disorders with an unclear phenotype[1][2,3]. Although exome and genome sequencing might be useful to identify genetic alterations in individuals with rare diseases, it is relevant to highlight that these techniques may also identify unexpected variants not related with the purpose of the primary analysis and they may come as a surprise for clinicians, patients and their families. One of the paradigmatic examples is the identification of pathogenic variants in cancer susceptibility genes (CSG).

An issue of debate in the medical genetics community is whether and how this unintended information should be reported to the patient and/or family. Recently, several international scientific societies have published a policy statement for managing SF/IF. While the European Society of Human Genetics (ESHG)[4,5] recommended that genetic analysis should be restricted to the first purpose and follow a cautious approach in regards to opportunistic screening, other societies like the French Society[6], the 100,000 Genomes Project Protocol[7], the British Society of Genomic Medicine, and the American College of Medical Genetics[8,9] (ACMG) have published different lists of genes, mainly comprising hereditary cardiopathies genes and CSG, for which it is recommended to report the secondary findings (SF). According to ACMG[8], incidental findings (IF) comprise those variants found unexpectedly, while secondary findings (SF) include

Little is known about the clinical and psychological impact of SF/IF on CSG in patients and their families. The main concerns comprise the potential harm derived from the uncertain penetrance in the absence of a family history, and the psychological distress resulting from identifying genetic alterations that are not the targets of the genetic study.

In this study, our main objective was to describe the clinical actionability and the psychological impact of SF/IF disclosure in CSG in patients and their relatives. Specifically, we aimed to: 1) evaluate the frequency of pathogenic variants in CSG as SF/IF among a cohort of patients undergoing clinical exome sequencing testing for a non-cancer condition, 2) analyze the uptake of predictive testing among family members, the adherence to screening and prevention recommendations, and the output of surveillance programs, and 3) compare the psychological impact of identifying pathogenic variants in CSG as SF/IF versus their identification in individuals with a cancer family history.

METHODS

Study design

Retrospective analysis of exome sequencing results ordered in 533 patients in the Department of Clinical and Molecular Genetics at University Hospital Vall d'Hebron, a tertiary care hospital, from January 1, 2018 to September 30, 2020. The psychological impact was analyzed with the Multidimensional Impact of Cancer Risk Assessment (MICRA) scale[10,11] within a case-control study design. Cases involved those individuals who were carriers of a SF/IF identified through exome sequencing, as well as their relatives, in the absence of any cancer history (n=53). Controls (n=576) were identified among individuals undergoing CSG testing due to personal or family history of cancer. Family history was designated as positive when there were first or second-degree relatives with a type of cancer associated to the CSG identified in the family

Genetic testing and counseling

Exome sequencing studies were ordered by health professionals of the Department of Clinical and Molecular Genetics to identify genetic causes for intellectual disability or congenital malformation, or to confirm a clinical suspicion of a syndrome not associated to cancer. Patients or their parents/guardians signed an informed consent and specified whether they opted-in to be disclosed SF(s). The IF was discussed in a multidisciplinary committee, which evaluated whether it should be disclosed based upon their penetrance and clinical actionability. Clinical recommendations for surveillance considered the overall health status of the carrier.

A SF related to CSG was defined as the identification of a pathogenic or likely pathogenic variant in a CSG listed by ACMG (ACMG SF V2.0)[8]. Cancer-associated genes with clinical actionability beyond this list were listed as IF. Variant interpretation was based on the ACMG guidelines[12]. The sequencing methodology is reported as supplementary material.

All participants underwent pre and post-test counseling with a board-certified genetic counselor. The project was approved by the local IRB.

Study Measurements

Clinical actionability of SF/IF was assessed by a review of the electronic medical records of those carriers in whom surveillance had been indicated, from the date that their result was disclosed until December 2021. The psychological impact was assessed by the Spanish version of the MICRA scale, which is a validated tool to measure the specific impact of result disclosure after genetic testing. The MICRA scale includes 25-items measuring general and cancer-specific distress, and it includes three main subscales: Distress (range 0-30) evaluates feeling upset, sadness, guilt, problems with enjoying life, and loss of control; Uncertainty (range 0-45) evaluates difficulties in making decisions, family conflict, and frustration; and the Positive Experiences subscale (range 0-20) evaluates relief, happiness and family communication. Carriers of a variant of uncertain significance, were excluded from the analysis of psychological impact. Satisfaction with the genetic counseling process, as well as the perceived feeling of regret for having disclosed an unexpected genetic finding were evaluated with three five Likert-type questions, with answers ranging from 0-10, or yes/no. Psychological impact and satisfaction with the genetic counseling process were analyzed at 3 months after results disclosure. Psychological impact was compared with a historical control population of individuals undergoing CSG testing due to their personal or family history at 3 months after results disclosure

Statistical analysis

The Mann–Whitney–Wilcoxon test was used to assess differences between cases and controls for the different MICRA sub-scales. Standardized difference was used to study the associations of clinical characteristics and MICRA results between cases and controls. The propensity score

weighting method was used to adjust for the imbalance in prognostic factors between cases and controls. We used ATT (average treatment effect among the treated method) to estimate the weights. The variables used for weighting the propensity scores were gender, age, study level, panel/exome or single pathogenic variant, positive or negative result. According to this approach, controls who were more similar to cases were those who had more influence in the analysis. Linear regression was used in order to evaluate which psychological impact (MICRA scale) variables had more impact in cases or controls. Statistical analyses were performed with R version 4.1.1.

RESULTS

Frequency of secondary findings in cancer susceptibility genes

Exome sequencing had been ordered in 582 individuals in the Clinical and Molecular Genetics Department of our institution. Exome testing had been performed during the prenatal period (n=6), the postnatal period (n=494), or shortly after pregnancy termination (n=33). A total of 8% (49/582) of the index cases opted-out to the disclosure of a SF/IF (Figure 1, CONSORT diagram). Overall, 11 index cases (IC) were found to carry a pathogenic variant in a CSG, which results in a 2.1% (95% CI 1.1% - 3.8%) frequency (11/533). Ten SF belonged to the ACMG V2.0 genes list, distributed among six genes – three in *BRCA2*, three in *PMS2*, two in *SDHB*, one in *BRCA1*, and one in *MLH1*. One IF was identified in the *RAD51C* gene. Eight cases (73%) were children, two were young adults with intellectual disability, and one was an adult (Table S1). Two index cases had a family history of breast cancer

Predictive genetic testing among relatives

Predictive genetic testing was performed in 42 relatives of the 11 index cases (Table S1). The median age was 47 years-old (range 6-78), with a 50% gender distribution. Among them, 23, 9

and 10 (54%, 22% and 24%) were first, second and third degree relatives, respectively. A pathogenic variant was identified in 18 (43%) relatives.

Clinical actionability in individuals with a pathogenic variant in a CSG

Overall, there were 29 carriers of a pathogenic variant in a CSG identified as SF/IF (11 index cases and 18 relatives), of which 20 enrolled in a cancer surveillance program and nine were excluded because of their age (Table S2). The Median time of surveillance was 24 months (range 12-30). Among the seven *SDHB* carriers, three (43%) had a paraganglioma that was diagnosed after genetic testing. A middle childhood 6 – 11 years *SDHB* male carrier was diagnosed with a multisite para-aortic paraganglioma in his first imaging screening and two women were diagnosed with a localized paraganglioma between the ages 40 and 60. There were seven *BRCA2* carriers (four men and three women). Among them, an early 70s woman who had ended the population-based breast cancer screening was diagnosed with a localized breast cancer after her initial imaging screening post genetic testing. Two other women underwent prophylactic salpingo-oophorectomy. One male with metastatic prostate cancer was candidate for targeted therapy with a PARP inhibitor after the identification of a *BRCA2* pathogenic variant. A woman with a pathogenic variant in the *RAD51C* gene underwent a prophylactic salpingo-oophorectomy. Among the three *PMS2* carriers, two had a negative colonoscopy, and one had two colonic adenomatous polyps removed.

Psychological impact

Within all cases (n=53), a total of 19 participants were excluded for this analysis (seven were younger than 16 years old, three had intellectual disability, one had a psychiatric disorder, one was a non-Spanish speaker and seven underwent genetic testing after the cut-off date). Among the 34

participants who were contacted to participate in this sub-study, 32 answered the MICRA scale (94%) (Figure 1). In all but one case the questionnaire was answered before their cancer diagnosis. The MICRA scale had previously been completed in the control population by 573 individuals

Comparison of the psychological impact with the MICRA scale between cases and controls

There were some significant differences in clinical characteristics between cases and controls (Table S3). Among the control population, the frequency of females was higher (73% vs 56%), the educational level was higher (67% vs 56%), the median age was older (median age 48 vs 46,5 years), and the proportion of carriers was 29% and 41%, respectively. The propensity model adjusted for these differences (Table S3).

The comparison between the MICRA scale scores in cases (n=32) and controls (n=573) is shown in Figure 2 and Table S4. The mean $\pm$ SD in the total MICRA was 21.18 ± 14.30 in cases vs 16.18 ± 12.54 in controls ($p=0.001$), in the distress subscale the mean $\pm$ SD was 6.31 ± 7.26 vs 4.16 ± 5.31 ($p=0.001$), and in the uncertainty subscale the mean $\pm$ SD was 8.31 ± 7.16 vs 6.58 ± 6.32 ($p=0.001$). The positive experience score was higher in controls than in cases (14.74 ± 5.18 vs 13.81 ± 4.82 , $p=0.001$).

In the univariable linear regression (Figure 3), cases maintained higher scores in the total MICRA and each of the negative feeling subscales; however, no differences were observed in the score of the positive experiences' subscale. Following the propensity score model, cases scored statistically significant higher values in the total MICRA scale (5.21 points above the mean of the controls), uncertainty (2.69 points above the mean of the controls) and distress (3.46 points above the mean of the controls) subscales. However, in the positive experience subscale the score was similar in both groups.

DISCUSSION

In the present study, the analysis of 533 exomes ordered for non-oncological diagnoses yielded a 2% cancer-related SF/IF. These findings led to 20 individuals enrolling in a cancer surveillance program, which prompted a cancer diagnosis in 20% of them in the first round. Overall, the psychological impact of SF/IF disclosure was not clinically relevant, although the scores were significantly higher in individuals receiving a SF/IF than in individuals receiving similar genetic results within a family with cancer history.

In this study, there was a 92% rate of acceptance for disclosure of SF/IF. This high rate highlights the importance of developing adequate delivery models of SF/IF disclosure to guarantee that those results turn into useful actions for the patients and their family members. The American College of Medical Genetics (ACMG) recommend offering participants an informed consent process and the option to opt out of SF/IF's disclosure[8]. Interestingly, in our study only 8% of the participants (or their legal representatives) chose to opt out for this type of disclosure, a slightly lower figure compared to a prior USA study that reported a non-acceptance in 17% of pediatric cases[13].

The 2% frequency of SF/IF in CSG in our study is in line with the frequency reported in other studies [14,15]. More specifically, the frequency of SF in a CSG was reported to range between 0.8% to 1.3%, mainly in the *BRCAl/2* genes[15,16]. We also found that pathogenic variants in *BRCAl/2* were the most frequent SF.

In our study, we extended the genetic testing to blood relatives at risk to promote surveillance and prevention options among potential carriers. Cascade testing was successfully performed in 42 relatives older than 16 years, apart from a family with an *SDHB* pathogenic variant, in whom genetic testing was also recommended for minors. Eighteen carriers of a pathogenic variant in a CSG among relatives of the index case were diagnosed. Most of the CSG conferred an increased

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3 risk of cancer in adulthood, except for one child with an *SDHB* pathogenic variant in whom a
4 metastatic paraganglioma was diagnosed in his first round. In all cases the predictive cascade
5 testing arrived to first degree relatives, in 55% families to second degree relatives, and in 45% to
6 third degree relatives. We highlight that there are members of the same families in both cases
7 and controls.

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2 It seems reasonable to hypothesize that these SF/IF findings might have a detrimental
3 psychological impact on patients and their relatives[17,18]. One of the concerns raised among
4 healthcare professionals is whether individuals who carry a pathogenic variant in a CSG without
5 cancer family history may underestimate the susceptibility to develop cancer and consequently
6 may not adhere to surveillance recommendations. However, in our study population, all carriers
7 adhered to the follow-up.

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the uncertainty and the distress after the genetic result were higher in those individuals in whom a SF/IF was identified compared to those from the control group, in whom a family history of cancer was triggering the genetic analyses. Feelings such as upset, sadness, guilt, indecision and frustration could be more frequently found in cases with unexpected cancer genetic findings in whom the genetic result is less expected and who are living within a rare disease. The psychological impact after cancer panel testing has largely been analyzed in several cancer cohorts[21–23]. Overall, the psychological impact is higher than in controls, but it is not ~~Genetic~~ Genetic counselors and geneticists should be cautious when conveying this information in the pre-test and post-test. This reinforces the need to guarantee genetic counseling in complex genetic analyses.

Given the increased psychological impact of SF/IF reporting in non-cancer families, future research should focus on developing strategies to minimise the impact of such results. It is crucial to emphasize the opt in/opt out decision of being disclosed SF/IF during the informed consent process in order to promote individual’s autonomy and provide an adequate counseling framework to enhance the understanding of the implications of SF/IF detection in index cases and healthy relatives.

LIMITATIONS

Some limitations of our study include: 1) Despite the size differences between two groups, we minimized this with the propensity weighting score model were those controls which were more similar to cases had higher weight in the statistical analysis. Moreover, both cases and controls could be members of the same families so this bias was present. In both groups. Although we mitigated imbalance in prognostic factors between cases and controls with the use of the propensity

score weighting method, there is still some imbalance between both groups that might impact the results; 2) the 3-month post disclosure follow-up for all participants might not provide sufficient time to manifest some of the psychosocial outcomes, despite our prior data suggests that psychological impact measured by MICRA does not change between 3- and 12-month post disclosure; 3) as primary and secondary findings were reported simultaneously, the distinction between them might not have been fully clear among study participants and thus generate a mixed impact in the MICRA score; 4) the number of cases was relatively small and this did not allow to provide precise estimations in this group.

CONCLUSION

The frequency of SF/IF in CSG was 2% in a large cohort of patients undergoing genetic testing due to non-oncological conditions without cancer family history. The identification of SF/IF after exome sequencing leads to the recognition of unaffected carriers at risk of developing cancer, with a high actionability as shown in our work. This demonstrates the importance of cascade testing in relatives. Our findings indicate that the participants who receive a SF/IF in CSG after exome sequencing have a numerically higher distress and uncertainty compared with those who undergo genetic testing due to a family history of cancer. Our study offers substantial insight for other clinicians who will need to address secondary findings and provide adequate genetic counseling. It also emphasizes the importance of the potential psychological effects of expanded genetic testing.

DATA AVAILABILITY

All data and methods used in the analysis are described or included in this article and in the electronic supplementary information. Raw data is available upon request.

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COMPETING INTEREST

J.B has received a speaker’s fee from AstraZeneca and Pfizer and has served AstraZeneca in an advisory role; G.V has received a speaker’s fee from MSD and Pierre Fabre and has served AstraZeneca in an advisory role.

L.M. is member of data monitoring committees for clinical trials sponsored by Novartis, Actuate Therapeutics, Shionogi, Incyte, the University of Southampton and the Royal Marsden NHS Foundation Trust; had a consulting role for Novartis, Norgine, Boehringer, Ymabs and Shionogi and participated in educational activities organized by Bayer and Eusa Pharma. L.M. is member of the Executive Committee of SIOPEN (European neuroblastoma research cooperative group), an organization which receives royalties for the sales of dinutuximab beta.

EFT has received grant support to conduct clinical trials on SMA from Ionis/Biogen and serves as

a consultant to Biogen, Novartis,AveXis, Roche, Biologix, and Cytokinetics.

GV has received research honoraria for speakers bureau from MSD and Pierre Fabrer and advisory role from Astrazeneca

The other authors declare no conflict of interest.

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We have not received any funding for this work

ETHICS DECLARATION

This study was reviewed by the Institutional Review Board of our institution. All individuals participating in the study or their parents/legal representatives signed the informed consent approved by the Institutional Review Board.

CONTRIBUTORSHIP STATEMENT

Conceptualization: E.C., J.B., L.M.; Data curation: E.C., S.T, MC, IV AMC, DP, AA; Formal Analysis: G.V., V.N., E.C.; Funding acquisition: J.B; Investigation: E.C., J.B., L.M.; Methodology: E.C., J.B., L.M., G.V., V.N.; Project administration: E.C.; Resources: A.D., S.T, M.C; C.S, O.D, D.P, A.C, I.V, B.P, E.FT, M.T, E.GA, Genetica.; Software: S.T.; G.V.; V.N;Supervision: J.B., L.M.; EGA, EFT;Validation: E.C., J.B., L.M.; Visualization: E.C., G.V; Writing: E.C; V.N; J.B; L.M.; ALF ;Writing review & editing: All authors .

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Figure 3. Distribution of MICRA scores scales and subscales between cases (n=32) and controls (n=576). Total MICRA score (range 0-105), Uncertainty (range 0-45), Distress (range 0-30), Positive experiences (range 0-20)

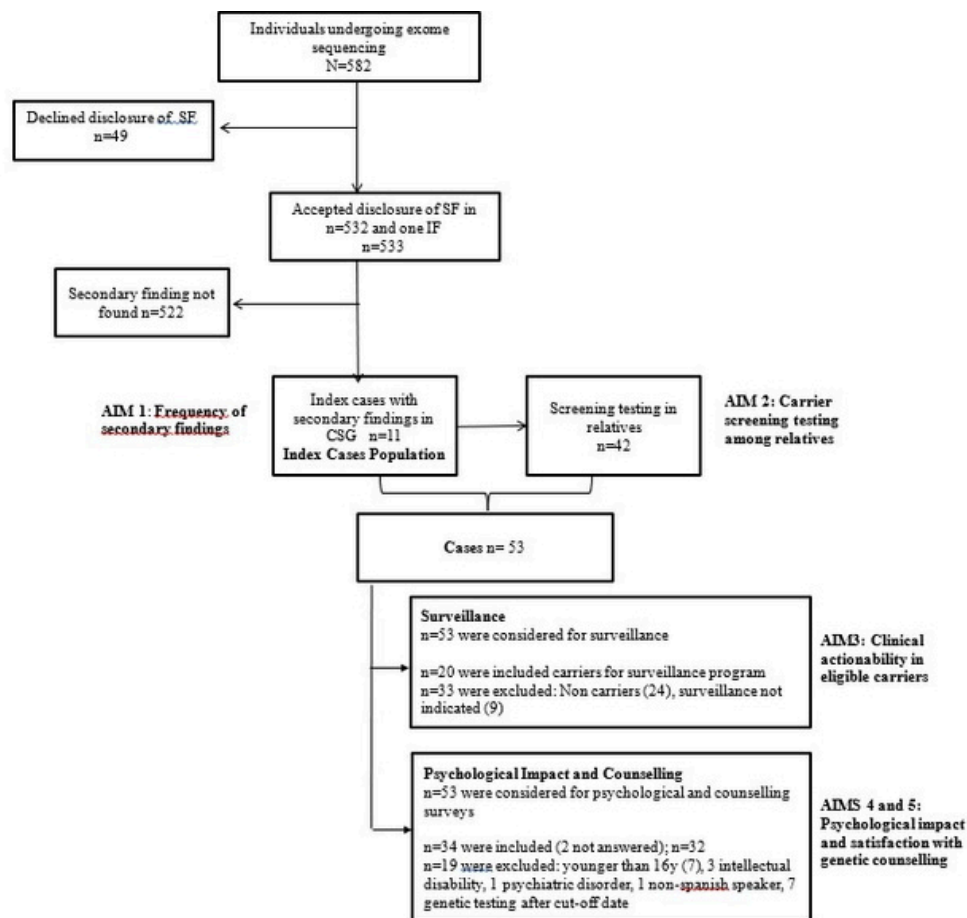


Figure1. CONSORT Diagram of study population

184x171mm (96 x 96 DPI)

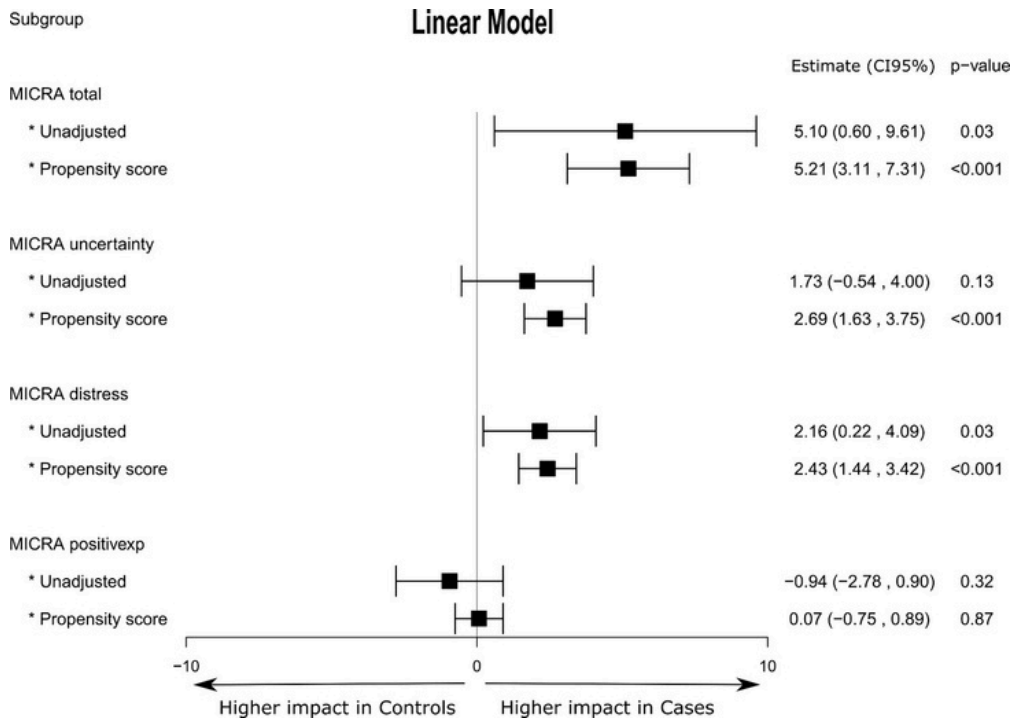


Figure 2. The mean difference in total MICRA and subscales scores between cases and controls. The X axis shows, 0=mean in controls; 5= 5 points above controls mean; 10= 10 points above controls mean; -5= 5 points below controls mean. Propensity score adjusted by gender, age, study level, exome and single pathogenic variant and carrier or non-carrier

321x227mm (96 x 96 DPI)

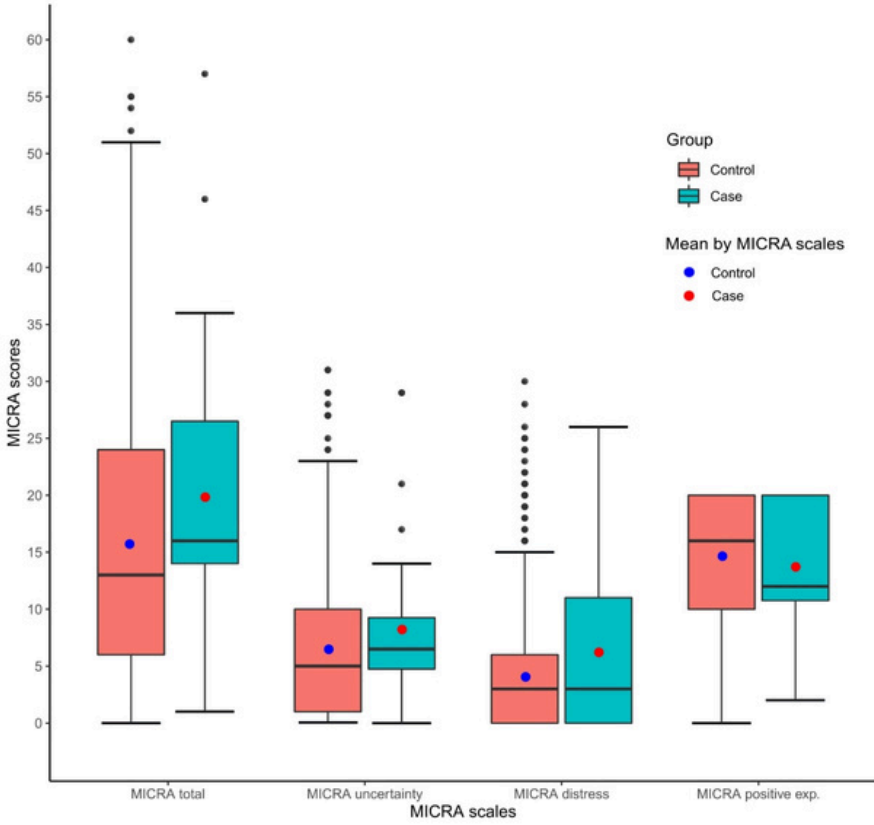


Figure 3. Total MICRA score (range 0-105), Uncertainty (range 0-45), Distress (range 0-30), Positive experiences (range 0-20)

584x486mm (87 x 87 DPI)

Supplementary Materials and Methods

Supplementary. Laboratory information

Databases consulted:

Single Database of Nucleotide Polymorphisms (dbSNP: <http://www.ncbi.nlm.nih.gov/SNP/>)

- 1000 Genomes (<http://www.1000genomes.org/>)
- NHLBI Exome Sequencing Project (ESP: <http://evs.gs.washington.edu/EVS/>)
- Exome Aggregation Consortium (ExAC: <http://exac.broadinstitute.org/>)
- Clinical variant (<http://www.ncbi.nlm.nih.gov/clinvar/>)
- The Human Gene Mutation Database Professional (HGMD: <http://www.hgmd.org/>)
- IMEGEN's own database

In silico prediction programs:

- SIFT (<http://sift.jcvi.org/>)
- PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>)
- Mutation Taster (<http://www.mutationtaster.org/>)
- PROVEAN human genome variants (http://provean.jcvi.org/genome_submit_2.php)
- LTR, SVM, FATHMM, GERP ++ score

The PMS2 gene has been analyzed by complete gene amplification (long PCR) and internal Sanger sequencing, following the methodology described in Vaughn et al 2010

Table S1. Description of index cases and relatives (n=53)

<i>ID</i>	<i>Gender</i>	<i>Age at genetic testing</i>	<i>Relative</i>	<i>Primary reason for testing</i>	<i>Primary genetic finding</i>	<i>CSG</i>
<i>P1-1</i>	M	30-35	Index case	Sotos phenotype	Carrier <i>DNMT3A</i> c.1681G>T	Carrier <i>SDHB</i> c.79C>T
<i>P1-2</i>	F	35-40	Sister	Cascade testing	NA	Non carrier <i>SDHB</i> c.79C>T
<i>P1-3</i>	F	60-65	Mother	Cascade testing	Non carrier <i>DNMT3A</i> c.1681G>T	Carrier <i>SDHB</i> c.79C>T
<i>P1-4</i>	M	60-65	Father	Cascade testing	Non carrier <i>DNMT3A</i> c.1681G>T	Non carrier <i>SDHB</i> c.79C>T
<i>P1-5</i>	F	55-60	Maternal aunt	Cascade testing	NA	Carrier <i>SDHB</i> c.79C>T
<i>P1-6</i>	F	40-45	Maternal Cousin	Cascade testing	NA	Carrier <i>SDHB</i> c.79C>T
<i>P1-7</i>	M	60-65	Maternal uncle	Cascade testing	NA	Non carrier <i>SDHB</i> c.79C>T
<i>B1-1</i>	F	0-5	Index case	Sturge-Weber	NF	Carrier <i>RAD51C</i> c.705+1G>A
<i>B1-2</i>	F	35-40	Mother	Cascade testing	NA	Carrier <i>RAD51C</i> c.705+1G>A
<i>B1-3</i>	M	25-30	Father	Cascade testing	NA	Noncarrier <i>RAD51C</i> c.705+1G>A
<i>B1-4</i>	F	60-65	Maternal GM	Cascade testing	NA	Carrier <i>RAD51C</i> c.705+1G>A
<i>B2-1</i>	F	0-5	Index case	Leukodystrophy	Carrier <i>GFAP</i> c.716G>A	Carrier <i>BRCA2</i> c.8847del
<i>B2-2</i>	M	40-45	Father	Cascade testing	Non carrier <i>GFAP</i> c.716G>A	Carrier <i>BRCA2</i> c.8847del
<i>B2-3</i>	F	45-50	Mother	Cascade testing	Non carrier <i>GFAP</i> c.716G>A	Non carrier <i>BRCA2</i> c.8847del
<i>B2-4</i>	F	45-50	Paternal Aunt	Cascade testing	NA	Non carrier <i>BRCA2</i>

						c.8847del
B2-5	F	45-50	Paternal Aunt	Cascade testing	NA	Carrier <i>BRCA2</i> c.8847del
B2-6	M	75-80	Paternal GP	Cascade testing	NA	Non carrier <i>BRCA2</i> c.8847del
B2-7	F	70-75	Paternal GM	Cascade testing	NA	Carrier <i>BRCA2</i> c.8847del
B2-8	M	65-70	Great uncle	Cascade testing	NA	Carrier <i>BRCA2</i> c.8847del
B2-9	M	35-40	Great uncle son	Cascade testing	NA	Non carrier <i>BRCA2</i> c.8847del
B2-10	F	35-40	Great uncle daughter	Cascade testing	NA	Non carrier <i>BRCA2</i> c.8847del
B3-1	F	30-35	Index case	Lymphedema and intestinal lymphangiectasia	Carrier <i>CELSR1</i> c.2451del (VUS)	Carrier <i>BRCA2</i> c.8847del c.3264dupT
B3-2	F	50-55	Mother	Cascade testing	Carrier <i>CELSR1</i> c.2451del (VUS)	Non carrier <i>BRCA2</i> c.3264dupT
B3-3	M	55-60	Father	Cascade testing	GTNI	Carrier <i>BRCA2</i> c.3264dupT
B3-4	M	25-30	Brother	Cascade testing	Carrier <i>CELSR1</i> c.2451del (VUS)	Non carrier <i>BRCA2</i> c.3264dupT
P2-1	M	5-10	Index case	Language delay, ID, small teeth, macrocephaly	Carrier <i>KDM5C</i> c.1510G>A	Carrier <i>SDHB</i> c.583_585del
P2-2	F	40-45	Mother	Cascade testing	Carrier <i>KDM5C</i> c.1510G>A	Carrier <i>SDHB</i> c.583_585del
P2-3	M	40-45	Father	Cascade testing	NA	Non carrier <i>SDHB</i> c.583_585del
P2-4	F	5-10	Sister	Cascade testing	NA	Carrier <i>SDHB</i> c.583_585del
P2-5	F	40-45	Maternal Aunt	Cascade testing	NA	Non carrier <i>SDHB</i> c.583_585del
P2-6	F	60-65	Maternal GM	Cascade testing	Carrier <i>KDM5C</i>	Non carrier <i>SDHB</i>

					c.1510G>A	c.583_585del
P2-7	M	65-70	Maternal GF	Cascade testing	NA	Non carrier <i>SDHB</i> c.583_585del
P2-8	M	35-40	Maternal uncle	Cascade testing	NA	Non carrier <i>SDHB</i> c.583_585del
C1-1	M	35-40	Index case	ID, peculiar phenotype, macrocephaly	NF	Carrier <i>PMS2</i> c.2175-2A>G
C1-2	F	70-75	Mother	Cascade testing	GTNI	Non Carrier <i>PMS2</i> c.2175-2A>G
C2-1	M	10-15	Index case	ID, Autism	Carrier <i>DNMT3A</i> c.2432_2434delinsC	Carrier <i>PMS2</i> c.137G>T
C2-2	F	45-50	Mother	Cascade testing	Non carrier <i>DNMT3A</i> c.2432_2434delinsC	Carrier <i>PMS2</i> c.137G>T
C2-3	M	45-50	Father	Cascade testing	Non carrier <i>DNMT3A</i> c.2432_2434delinsC	Non Carrier <i>PMS2</i> c.137G>T
C2-4	F	70-75	Maternal GM	Cascade testing	NA	Non Carrier <i>PMS2</i> c.137G>T
C2-5	M	35-40	Maternal uncle	Cascade testing	NA	Non Carrier <i>PMS2</i> c.137G>T
C2-6	M	45-50	Maternal uncle	Cascade testing	NA	Non Carrier <i>PMS2</i> c.137G>T
B4-1	M	20-25	Index case	ID, peculiar phenotype, cryptorchidism	NF	Carrier <i>BRCA2</i> c.1813dup
B4-2	F	55-60	Mother	Cascade testing	NA	Non carrier <i>BRCA2</i> c.1813dup
B4-3	M	55-60	Father	Cascade testing	NA	Carrier <i>BRCA2</i> c.1813dup
B4-4	M	70-75	Paternal uncle	Cascade testing	NA	Carrier <i>BRCA2</i> c.1813dup Carrier <i>BRCA1</i>
B5-1	F	0-5	Index Case	Noonan syndrome	Carrier <i>PTPN11</i> c.1510A>G	c.5309G>T Carrier <i>BRCA1</i>
B5-2	M	40-45	Father	Cascade testing	Carrier <i>PTPN11</i> c.1510A>G	c.5309G>T

C3-1	F	10-15	Index Case	ID, psychomotor and language delay	NF	Carrier <i>MLH1</i> c.306+5G>A
C3-2	F	45-50	Mother	Cascade testing	NA	Carrier <i>MLH1</i> c.306+5G>A
C3-3	M	45-50	Father	Cascade testing	NA	Non carrier <i>MLH1</i> c.306+5G>A
C4-1	F	Prenatal	Index Case	Prominent filtrum, proptosis, long bones, polyhydramnios,	Carrier <i>COL11A1</i> c.3816+2_3816+3insTT	Carrier <i>PMS2</i> c.989-2A>G
C4-2	M	35-40	Father	Cascade testing	Non Carrier <i>COL11A1</i> c.3816+2_3816+3insTT	Carrier <i>PMS2</i> c.989-2A>G
C4-3	F	30-35	Mother	Cascade testing	Non Carrier <i>COL11A1</i> c.3816+2_3816+3insTT	Non Carrier <i>PMS2</i> c.989-2A>G

IC: Index cases; ID: Intellectual Disability; NF: Not found; NA: not applicable (de novo mutation in index case and carrier

screening not indicated)

TableS2. Clinical actionability in eligible carriers of a pathogenic variant in a CSG (n=20)

<i>ID</i>	<i>Gender</i>	<i>Age at genetic testing</i>	<i>Relationship with index case</i>	<i>CSG</i>	<i>Cancer Susceptibility Syndrome</i>	<i>Recommended surveillance</i>	<i>Clinical findings during surveillance</i>
<i>P1-1</i>	M	30-35	IC	<i>SDHB</i>	<i>Syndromic PRG and PHEO #115310</i>	Biochemical studies and imaging	Normal findings
<i>P1-3</i>	F	60-65	FDR	<i>SDHB</i>	<i>Familial PRG and PHEO #115310</i>	Biochemical studies and imaging	Elevated methanephrynes in urine
<i>P1-5</i>	F	55-60	SDR	<i>SDHB</i>	<i>Familial PRG and PHEO #115310</i>	Biochemical studies and imaging	Abdominal PRG
<i>P1-6</i>	F	40-45	TDR	<i>SDHB</i>	<i>Familial PRG and PHEO #115310</i>	Biochemical studies and imaging	Cava inter-aortic PRG
<i>B1-4</i>	F	60-65	SDR	<i>RAD51C</i>	<i>Hereditary (Breast) and Ovarian Cancer # 613399</i>	Ovarian risk reduction surgery	<i>PBSO</i> : negative findings
<i>B2-2</i>	M	40-45	FDR	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer # 612555</i>	Biochemical and prostate imaging	Normal Findings
<i>B2-5</i>	F	45-50	SDR	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer # 612555</i>	Breast imaging and risk reduction surgery	Breast normal findings <i>PBSO</i> : negative findings
<i>B2-7</i>	F	70-75	SDR	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer # 612555</i>	Breast imaging and risk reduction surgery	MRI: breast cancer <i>PBSO</i> : negative findings
<i>B2-8</i>	M	65-70	Other	<i>BRCA2</i>	<i>Hereditary Breast and</i>	Biochemical and prostate imaging	Normal Findings

					<i>Ovarian Cancer</i> # 612555		
<i>B3-1</i>	F	30-35	IC	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer</i> # 612555	Breast imaging and risk reduction surgery	Normal findings No preventive surgeries
<i>B3-3</i>	M	55-60	FDR	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer</i> # 612555	Biochemical and prostate imaging	Normal findings
<i>P2-1</i>	M	5-10	IC	<i>SDHB</i>	<i>Familial PRG and PHEO</i> #115310	Biochemical studies and imaging	Para-aortic PRG
<i>P2-2</i>	F	40-45	FDR	<i>SDHB</i>	<i>Familial PRG and PHEO</i> #115310	Biochemical studies and imaging	Normal findings
<i>P2-4</i>	F	5-10	FDR	<i>SDHB</i>	<i>Familial PRG and PHEO</i> #115310	Biochemical studies and imaging	Normal findings
<i>C1-1</i>	M	35-40	IC	<i>PMS2</i>	<i>Lynch Syndrome</i> # 614337	Colonoscopy	Normal findings
<i>C2-2</i>	F	45-50	FDR	<i>PMS2</i>	<i>Lynch Syndrome</i> # 614337	Colonoscopy and gynecological examination	2 polyps (low grade tubular adenomas) Endometrial normal findings
<i>B4-3</i>	M	55-60	FDR	<i>BRCA2</i>	<i>Hereditary Breast and Ovarian Cancer</i> # 612555	Biochemical and prostate imaging	Negative biopsy after PIRADS3 radiological finding
<i>B4-4</i>	M	70-75	FDR	<i>BRCA1</i>	<i>Hereditary Breast and Ovarian Cancer</i> # 612555	Biochemical and prostate imaging	Normal findings

C3-2	F	45-50	FDR	MLH1	Lynch Syndrome # 609310	Colonoscopy and transvaginal ultrasound exam	Normal findings Recent Breast cancer diagnosis
C4-2	M	35-40	FDR	PMS2	Lynch Syndrome # 614337	Colonoscopy	Normal findings

ID: Identification; CSG: Cancer Susceptibility gene; IC: Index case; FDR: 1st degree; SDR: 2nd degree; PRG: Paraganglioma; PHEO: Pheochromocytoma; PBSO: Prophylactic Bilateral Salpingo-oophorectomy; MRI: magnetic resonance imaging. PIRADS3: Prostate Imaging Reporting and Data System, (3=the presence of clinically significant cancer is equivocal); # OMIM number .

Table S3:Baseline characteristic of controls in unweighted and weighted model

	<i>Unadjusted comparison</i>			<i>Adjusted comparison</i>		
	Cases (n=32)	Controls (n=576)	Standardized difference	Cases (n=32)	Controls (n=576)	Standardized difference
Gender						
Female	18 (56.2%)	422 (73.3%)	0.362	56.2%	55.71%	0.011
Male	14 (43.8%)	154 (26.7%)		43.8%	44.29%	
Age, median (IQR)	46.5 (40 –62.3)	48 (40-57)	0.103	46.5 (40 –62.3)	49 (40-61)	0.103
Study level						
Upto secondary school	14 (43.8%)	189 (32.8%)	0.223	43.8%	43.52%	0.005
Highschool or more	18 (56.2%)	387 (67.2%)		56.2%	56.48%	
Panel/Single PV						
Panel	1 (3.1)	291 (50.5%)	1.266	3.1%	3.13%	0.002
Single PV	31 (96.9)	285 (49.5%)		96.9%	96.87%	
Result						
Negative	19 (59.4%)	406 (70.5%)	0.234	59.4%	59.37%	<0.001
Positive	13 (40.6%)	170 (29.5%)		40.6%	40.63%	

**Differences between cases and controls. In the adjusted comparison adjusting by gender, age, study level, panel-exome or single PV, carrier or non-carrier, each controls gets a weight in the analysis. Each case weight had 1 value, those controls more similar to cases had a highest weight close to 1 value. in the study. PV: Pathogenic variant*

Table S4. Description of MICRA scores in cases and controls

	Controls n= 576			Cases n=32		
MICRA scale	Median	Mean	SD	Median	Mean	SD
MICRA_distress	3	4.156	5.315	3	6.31	7.38
MICRA_uncert	5	6,58	6,32	7	8.31	7.21
MICRA_positivexp	16	14,76	5,18	12	13.81	4.895
MICRA_total	13	16,18	12,54	17	21.28	14.53

RESPONSE TO REVIEWERS

Thank you for considering our manuscript "**Clinical and psychological implications of secondary and incidental findings in cancer susceptibility genes after exome sequencing in patients with rare disorders**" for publication in Journal of Medical Genetics and requesting a revised version.

We also thank the reviewers for their valuable comments. We have revised the manuscript according to these suggestions and addressed the specific questions. Please find hereunder our response to the comments, with the comment appearing in black text and our reply following in bold text. The revised manuscript has been uploaded in two forms, one clean and another with track changes, as detailed in the instructions to authors.

RESPONSE TO REVIEWERS

Reviewer: 1

Comments to the Author

Thank you for the invitation to review this paper. This is a novel and timely study and I really enjoyed reading the manuscript. The subject of IF/SF has been raised frequently through local and national MDT in recent months. The manuscript is well written and I particularly like how the impact on the wider family has been evaluated as well as the follow up and outcomes of the surveillance in family members who had inherited the pathogenic variant.

We are grateful for the careful review of our submitted manuscript.

A few small comments to make

-In first line of summary box the line starting "Nevertheless.." needs amending as does not read correctly.

Thank you, this sentence has been rewritten.

-Page 7 - another concern that could be highlighted is identification of a CSG in a child who is having genetic investigation for severe developmental delay. As well as the psychological impact, there is also the consideration of practicalities and ethics of surveillance in a child who has severe learning disability or other co-existing medical problems and how this might best be performed - this situation can pose challenges in clinical practice.

Thank you for raising this challenging clinical situation. We added a sentence in page 6 (genetic testing and counseling) to highlight this clinical situation

-Page 8 -how were the cancers that are related to the CSG decided -this is not always straightforward- was this expert opinion?

We followed robust associations of cancer and germline susceptibility according to published data on cancer-risk penetrance for each gene.

-In the study pre and post-test counselling was provided by a GC. However, requesting exome and genome testing is moving to mainstream clinicians who will undertake the consent and initial return of results. Consent is highlighted as an important part of the process. It would be helpful to know if any of the tests were ordered from mainstream specialist and if not, in the discussion,

We agree with the reviewer that mainstreaming exome or genome testing might increase even more the complexity of providing an adequate genetic counseling. In our research study, all individuals underwent pre- and post-test counseling by a genetic counselor or a clinical geneticist, which might have mitigated the risk of psychological distress. Despite the adequate framework of genetic counseling by professionals with certified communication skills, the scores of psychological impact were numerically higher in participants with secondary/incidental findings than in controls. Consequently, we believe that disclosure of secondary/incidental findings cope with the risk of causing more distress than primary findings in the context of personal or family criteria. Therefore, we claim the need to guarantee an adequate genetic counselling support in complex genetic analyses (Discussion page 13)

We have added the extent of relationship in the results (page 8-9)

Correct, there was a misspelling

Reviewer: 2

This is both an interesting and novel study, with findings of very high clinical relevance. While the study findings are highly novel, additional limitations in sample size, study design, and context should be acknowledged. Specific feedback included below.

We highly appreciate the positive feedback of the reviewer with the objective criticism to improve the quality of the manuscript

The authors discuss this as a case control study, however the 2 groups are not directly comparable; and there are some differences in characteristics across these 2 groups – this should be acknowledged in the discussion/limitations, and how this could impact results should also be explained.

The reviewer brings up a good point. Initially, the 2 groups are not comparable due to differences in baseline characteristics. For that reason, we used the propensity score weighting method to correct for the imbalance. The method mitigated these differences to obtain groups with similar characteristics, that could be compared (Supplementary Table 3). However, we agree that there are still some imbalances that could impact the results. The following sentence has been added in the limitation section:

“although we mitigated imbalance in prognostic factors between cases and controls with the use of the propensity score weighting method, there is still some imbalance between both groups that might impact the results”

2. The sample size of cases is very small and quite heterogeneous – again, should be acknowledged, and explain how these limits the strength of findings, etc.

We agree with the reviewer that the sample size of cases is quite small and that present a clear limitation of the study. Smaller sample sizes are associated with a lack of statistical power to detect real difference. On one hand, this study does not have a problem with lack of statistical power because differences in MICRA scores were detected even with the small size (mainly because differences in MICRA values seem to be large and consistent between cases and controls). On the other hand, it is true that the small sample size did not allow to provide precise estimations in this group. This limitation has been added in the current version of the manuscript.

“The number of cases was relatively small and this did not allow to provide precise estimations in this group”.

There was quite a difference between positive results in cases, and those in whom surveys were obtained – again, authors should acknowledge this limitation, and explain how this could have impacted their results.

The positive result was one of the variables used to adjust the imbalance between cases and controls. Initially the percentage of positive results for cases and controls was 40.6% and 28.5% respectively. The adjusted analysis allocated more weight in the control group with positive results to create a more comparable population (40.6% vs 40.63%) (Supplementary Table 3). Based on this, the difference in the MICRA results should not be impacted for the percentage of positive results.

The authors discuss cascade testing that was done, and high rate of uptake – this portion of the study is very much context dependent in terms of how it was done, how it was paid for, and what support was received – while the focus of this study is not on the uptake of cascade testing, how this facet of the study could have impacted results (given that relatives who tested positive were also counted as ‘cases’) should be discussed; and also given that there is potential for confounding when multiple cases are related (from the same family); compared to the controls, where it is unclear that any of the controls were related, or were included based on cascade testing. This point needs some discussion, and also included in the limitations section, given there is potential for it to have impacted results.

This study was done in a public hospital within a national health system. Participants did not have to pay for their clinical management nor received any payment to participate in the research study. Both cases and controls included relatives of the proband, therefore the same bias is shared in both groups. We have added this point in the discussion (page 12).

Minor:

5. There are grammatical errors that should be reviewed/corrected throughout.

To improve the quality of the manuscript, we have sent it for revision to a native English teacher.