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ANATOMICAL PATHOLOGY

Significant association between *FGFR1* mutation frequency and age in central giant cell granuloma

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Summary

Central giant cell granulomas (CGCG) are rare intraosseous osteolytic lesions of uncertain aetiology. Despite the benign nature of this neoplasia, the lesions can rapidly grow and become large, painful, invasive, and destructive. The identification of molecular drivers could help in the selection of targeted therapies for specific cases. *TRPV4*, *KRAS* and *FGFR1* mutations have been associated with these lesions but no correlation between the mutations and patient features was observed so far.

In this study, we analysed 17 CGCG cases of an Italian cohort and identified an interesting and significant (p=0.0021) correlation between *FGFR1* mutations and age. In detail, *FGFR1* mutations were observed frequently and exclusively in CGCG from young (<18 years old) patients (4/5 lesions, 80%). Furthermore, the combination between ours and previously published data confirmed a significant difference in the frequency of *FGFR1* mutations in CGCG from patients younger than 18 years at the time of diagnosis (9/23 lesions, 39%) when compared to older patients (1/31 lesions, 0.03%; p=0.0011), thus corroborating our observation in a cohort of 54 patients.

FGFR1 variants in young CGCG patients could favour fast lesion growth, implying that they seek medical attention earlier. Our observation might help prioritise candidates for *FGFR1* testing, thus opening treatment options with FGFR inhibitors.

Key words: Central giant cell granuloma; *FGFR1*; mutation frequency; age.

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INTRODUCTION

Central giant cell granulomas (CGCG), also known as central giant cell lesion of the jaw (CGCLJ), are rare intraosseous osteolytic lesions of uncertain aetiology that represent around 10% of all benign neoplasms of the jaws. They have a peak incidence in the second decade of life (34% of all cases), they appear more frequently in females than in males (with 60% of cases occurring in females) and are usually more often located in the mandible than in the maxilla (with 70% of cases occurring in the mandible) (Supplementary Table 1, Appendix A). Histologically, the lesion is composed of highly cellular stroma with spindle-shaped mononuclear cells and the pathognomonic multinucleated giant cells, usually located around areas of haemorrhage.[1,2] Despite the benign nature of this neoplasia, the lesions can rapidly grow and become large, painful, invasive, and destructive.

Surgical procedures span from conservative, such as enucleation and/or curettage, to extensive procedures such as *en bloc* resection. The latter are associated with a lower recurrence rate but may result in higher morbidity and the need for secondary reconstructive operations. Conservative surgery together with pharmacological treatment, including the antiangiogenic agent interferon alpha or corticosteroids, have been used as an alternative; however, these approaches present several drawbacks including adverse effects related to

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the pharmacological treatments. Identification of molecular drivers could be of great importance for the selection of targeted therapies.

A recent genomic characterisation of CGCG/CGCLJ revealed that *TRPV4*, *KRAS* and *FGFR1* mutations drive central CGCG, and activate the MAPK pathway[3] suggesting that some CGCG patients could benefit from MEK inhibitors or therapies targeting *TRPV4* and *FGFR1*. However, in that study no correlation between the mutations and patient features was observed.

In order to further explore the genetics of this neoplasia, and to possibly identify correlations between molecular drivers and patient features, we analysed CGCG in an Italian cohort consisting of 17 patients (5 patients <18 years old and 12 patients >18 years old) from three different Italian institutions. We performed whole exome sequencing (WES) of 10 cases and target analysis with Sanger sequencing of the additional seven cases (Supplementary Table 2; Appendix A).

MATERIALS AND METHODS

Sample collection and DNA extraction

CGCG/CGCLJ samples were obtained from formalin-fixed, paraffin-embedded (FFPE) tissue blocks from the surgical pathology files of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy), Istituto Stomatologico Italiano (Milan, Italy), and Azienda Ospedaliero-Universitaria di Parma (Parma, Italy), with informed consent from the patients and ethical approval from institutional and local research committee boards (Comitato Etico Milano Area 2, Milan, Italy).

Prior to the study, all patient samples were anonymised and used in alliance with the ethical rules and regulations presented in the Declaration of Helsinki. In total, 24 cases with the diagnosis of CGCG/CGCLJ were identified. Information for all cases was acquired during clinical appointments or retrieved from patient files (AV, NF, AC and AEB). All H&E slides were revised by bone pathologists (EA, AD, AP) to confirm diagnosis.

DNA from FFPE tissues (7×10 µm sections) was extracted and purified using the AllPrep DNA/RNA FFPE Kit (Qiagen, Germany), following the manufacturer's instructions. The DNA was quantified using NanoDrop ND 1000 Spectrophotometer (ThermoFisher Scientific, USA). Seventeen CGCG DNA samples were selected based on sufficient DNA yield and quality (260/280 >1.7 and possibility to amplify 200 bp amplicons).

Clinicopathological information of the 17 selected cases are reported in Table 1 and Supplementary Table 2 (Appendix A). Upon informed consent, a sample of an oral swab was collected prospectively during routine medical check-ups for nine of the 17 selected patients, as indicated in Supplementary Table 2 (Appendix A). Control DNA was extracted, purified and amplified using QIAamp and REPLI-g Midi Kit (Qiagen) using standard protocols.

Table 1 Clinicopathological features of Italian central giant cell granuloma (CGCG) cohort

Variable	*n* (%)
Gender	
Female	10 (58.8%)
Male	7 (41.2%)
Age	
<18	5 (29.4%)
>18	12 (70.6%)
Tumour site	
Maxilla	7 (41.2%)
Mandible	10 (58.8%)
Recurrence during study	
Yes	5 (29.4%)
No	12 (70.6%)
Post-operative interferon alpha treatment	
Yes	4 (23.5%)
No	13 (76.5%)

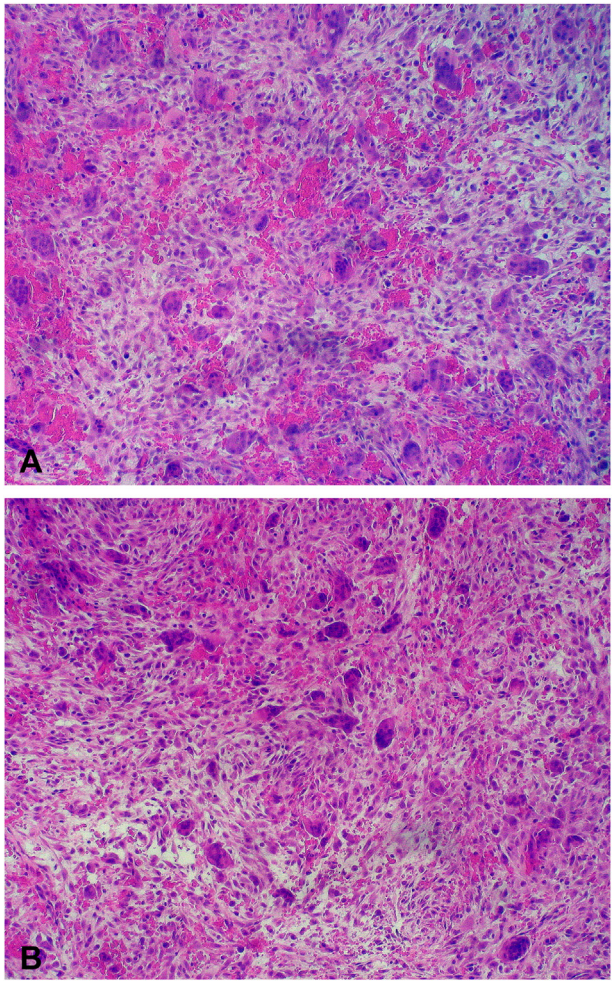

Fig. 1 H&E staining of two CGCG cases: (A) Case #4, 14-year-old patient, and (B) Case #7, 23-year-old patient, exhibiting clusters of relatively sparse and randomly scattered multinucleated giant cells embedded in a stroma of oval to spindle-shaped mononuclear cells.

WES library preparation and NovaSeq sequencing

Initial DNA sample quality assessment, DNA library preparations and sequencing were conducted at Genewiz (Germany). The genomic DNA was quantified using the Qubit 4.0 Fluorometer (Invitrogen, USA) and qualified using the Agilent 5300 Fragment Analyzer (Agilent, USA). Six CGCG samples were selected based on control DNA availability at the time of WES. An additional four CGCG samples with sufficient DNA quantity/quality were also processed. Enrichment probes were designed against the region of interest and synthesised through Agilent Technologies (USA). Library preparation was performed according to the manufacturer's guidelines. Briefly, the genomic DNA was fragmented by acoustic shearing with a Covaris E220 instrument (Covaris, USA). Fragmented DNA was end repaired and adenylated at the 3′ ends. Adapters were ligated to the DNA fragments, and adapter-ligated DNA fragments were enriched with limited cycle polymerase chain reaction (PCR). Adapter-ligated DNA fragments were validated using Agilent Fragment Analyzer (Agilent Technologies), and quantified using Qubit 2.0 Fluorometer. Adapter-ligated DNA fragments were hybridised with biotinylated baits. The hybrid DNA was captured by streptavidin-coated binding beads. After extensive washing, the captured DNA was amplified and indexed with indexing primers (Illumina, USA). Post-captured DNA libraries were validated using the Agilent 5300 Fragment Analyzer and quantified using the Qubit 2.0 Fluorometer. Illumina reagents and kits for DNA library sequencing cluster generation and sequencing were used for enrichment DNA sequencing. Post-captured libraries were multiplexed on a flowcell and loaded on the NovaSeq 6000 instrument (Illumina) according to the manufacturer's instructions. The

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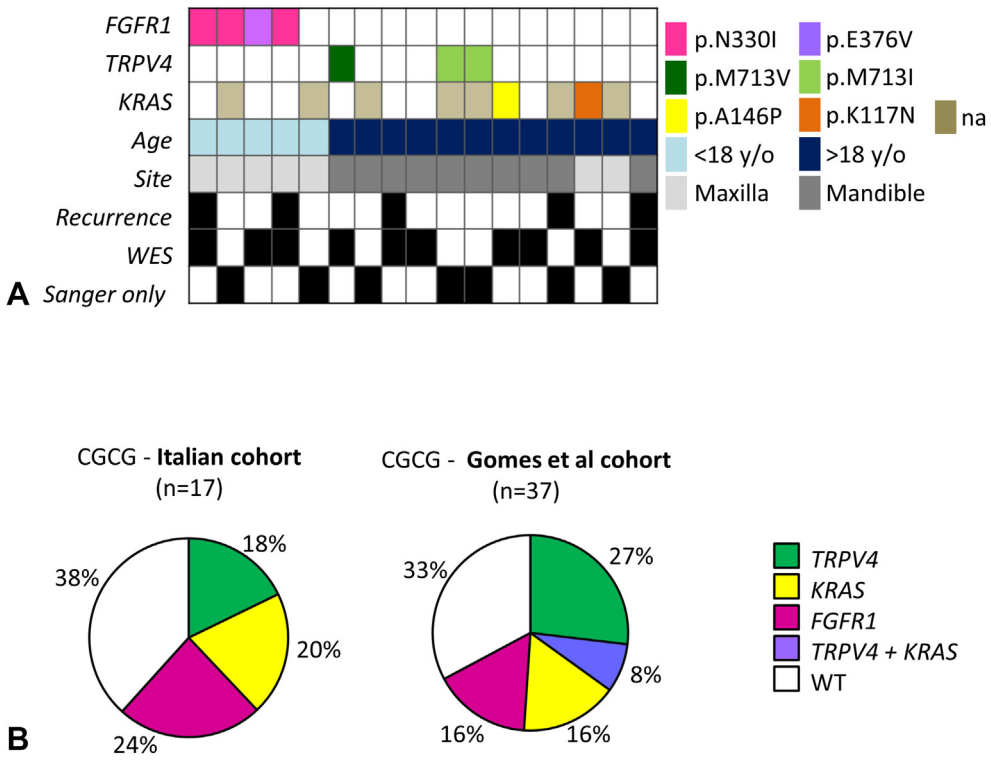

Fig. 2 (A) Summary of *TRPV4*, *KRAS*, and *FGFR1* mutations identified in the Italian cohort. (B). Pie-chart showing the percentage of *TRPV4*, *KRAS*, and *FGFR1* mutations in the Italian cohort (left), and in Gomes *et al.*[3] cohort (right).

samples were sequenced using a 2x150 paired end (PE) configuration. Image analysis and base calling was conducted by the NovaSeq Control Software on the NovaSeq instrument. Raw sequencing data (.bcl files) generated from NovaSeq were converted into fastq files and de-multiplexed using Illumina's bcl2fastq software.

Data analysis

Sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic 0.39.[4] Cleaned reads were then aligned to the GRCm38 reference genome using Sentieon 202112.[5] Alignments were then sorted, and PCR/optical duplicates were marked. BAM files were generated at this step. Somatic single nucleotide variants (SNVs) and small indels were called using Sentieon 202112 (TNSeq algorithm).[5] The VCF files generated by the pipeline were then normalised (left alignment of indels and splitting multiallelic sites into multiple sites) using bcftools 1.13.[6] Overlapped transcripts were identified for each variant and the effects of the variants on the transcripts were predicted by Ensembl Variant Effect Predictor (VEP) v104.[7] The most severe impact was selected for each variant, and they were used for downstream cohort analysis. Impact of the variants was also classified based on Mutation Annotation Format (MAF) document specifications.[8] A series of filtering and prioritisation steps were followed to identify genuine somatic and pathogenic variants. Only non-synonymous SNVs (missense, splice site, nonsense) and indels with $\geq$15% variant allele frequency were prioritised. SIFT[9] and PolyPhen[10] bioinformatic tools were utilised to predict the potential pathogenic effect of missense substitutions and only variants that were predicted 'deleterious/damaging' by at least one tool were retained. Variants were manually visualised and investigated using Integrative Genomics Viewer (IGV).[11] Variants were retained if they were novel or pathogenic (1000Genomes, gnomAD, COSMIC, ClinVar).

Sanger sequencing

DNA was amplified by PCR targeting the genomic regions flanking the variant. PCR products were purified using microCLEAN (Microzone, UK). Sequencing reactions were performed using the BigDye v3.1 cycle sequencing kit and sequenced on an ABI 3730 DNA analyser (Applied Biosystems, USA), following manufacturer's guidelines.

Additional statistical analysis

Fisher's exact test (two-sided) and multivariate analysis (Correlation matrix, Pearson correlation coefficient) were performed using Graphpad Prism version 8 (GraphPad, USA).

RESULTS AND DISCUSSION

In this study we analysed CGCG from an Italian cohort consisting of 17 patients (five patients <18 years old and 12 patients >18 years old) from three different Italian institutions. Clinical information is reported in Table 1 and Supplementary Table 2 (Appendix A), while histological images of two representative CGCG cases are shown in Fig. 1.

To improve the understanding of CGCG molecular drivers, WES was conducted on 10 cases (6 normal paired tumours and 4 tumours only). Point mutations were identified in *TRPV4*, *KRAS* and FGFR1genes as previously reported by Gomes *et al.*[3] Target analysis (Sanger sequencing) of the additional seven cases was then performed. *FGFR1, KRAS* and *TRPV4* were mutated in 24%, 20% and 18% of cases, respectively (Fig. 2), consistent with the mutation frequency identified in the Brazilian cohort (Fig. 2B). In our samples, we did not detect *TRPV4* and *KRAS* co-occurring mutations that were identified in three cases of the Brazilian cohort; this is probably due to the smaller analysed cohort.

In order to explore any possible association between *FGFR1*, *KRAS* or *TRPV4* mutations and clinical features, we performed multivariate analyses (Fig. 3). The results indicated a significant association between the *FGFR1* mutation status and both age and location, in the Italian cohort (Fig. 3A). The association between *FGFR1* mutations and age was also confirmed by analysing the two cohorts together (combined cohort, Fig. 3B). Regarding *KRAS* and *TRPV4* we

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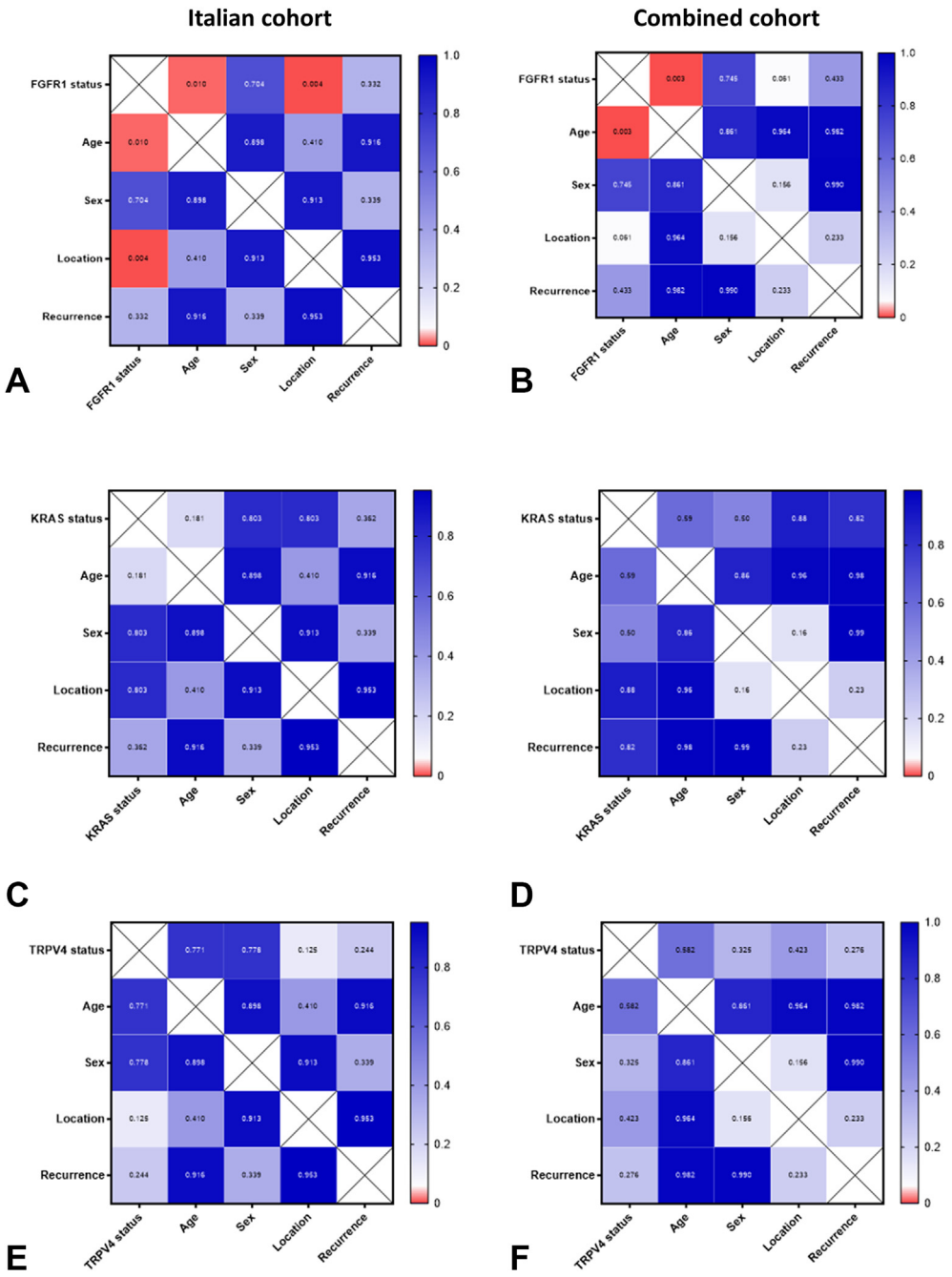

Fig. 3 Correlation matrix displaying the correlations between *FGFR1* (A,B), *KRAS* (C,D), or *TRPV4* (E,F) mutations and patients' clinicopathological features in the Italian cohort (A,C,E) and in the combined cohorts (B,D,F) cohorts. *p* values are indicated for each correlation.

could not identify any association between the mutation profile and clinicopathological features, in the Italian cohort (Fig. 3C,E, respectively), or in the combined cohort (Fig. 3D,F, respectively). These results confirm the previous findings by Gomes *et al.*[3]

Considering the association between young age and *FGFR1* mutations we decided to further analyse this aspect by setting a cut-off age of 18 years, considering paediatric patient definitions of the National Institutes of Health (NIH, USA) and National Health Service (NHS, UK).

In our cohort, *FGFR1* mutations were frequent (4/5 lesions, 80%) in patients <18 years and were not identified in patients >18 years (n=12). A significant difference was noticed in the frequency of *FGFR1* mutations in CGCG from patients <18 years versus >18 years at diagnosis (p=0.0021) (Fig. 4, upper panel).

In the Italian cohort, the previously identified nucleotide substitution leading to p.N330I was identified in three tumours while a novel somatic mutation leading to p.E376V was detected in one case. The difference in *FGFR1* mutation frequency according to patient age remained significant even considering only the known N330I gain-of-function mutation (p=0.0147). No variants leading to p.C381R were observed in our cohort. In the Gomes *et al.* cohort, an association between *FGFR1* mutations and age could be also perceived, even though to a minor extent (p=0.0897) (Fig. 4, middle

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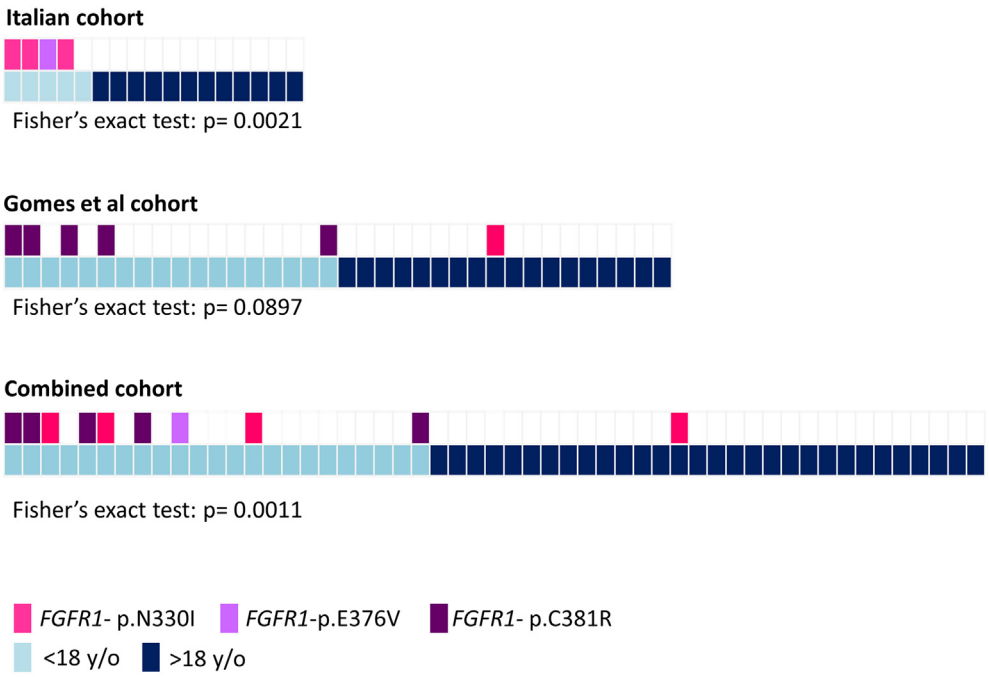

Fig. 4 Distribution of *FGFR1* somatic mutations in CGCG. *FGFR1* mutations were significantly associated with patient age (<18 years old) in the Italian and the combined cohort (Fisher's exact test, two-sided, was calculated with GraphPad Prism version 8). Each rectangle represents one tumour sample. Purple rectangles denote *FGFR1* mutations. Light blue and blue rectangles indicate patient age.

panel). The combination of the two sets of data confirmed a significant difference in the frequency of *FGFR1* mutations in CGCG from patients <18 years (9/23 lesions, 39%) versus patients >18 years at diagnosis (1/31 lesions, 0.03%; p=0.0011) (Fig. 4, lower panel), thus corroborating our observation in a cohort of 54 patients.

Differences in driver genes according to patient age have been reported extensively in many tumour types.[12,13] In young individuals, specific genomic drivers could lead to a more rapid development of the tumour. Accordingly, *FGFR1* variants in young CGCG patients could favour fast growth of the lesion, thus implying that they seek medical attention earlier. We did not observe differences in CGCG aggressiveness nor recurrence based on the presence of *FGFR1* mutations. However, it is worth mentioning that de Lange *et al.* identified a correlation between young age and higher recurrence rate in male CGCG patients.[14] Currently, no association between *FGFR1* mutations and patient features, other than age, can be appreciated by analysis of the combined data sets. Nonetheless, in the Italian cohort, *FGFR1* mutations were also associated with maxilla CGCG (p=0.0147, Fisher's exact test). This is probably linked to the unusual association between young age and CGCG location in our set (p=0.0034, Fisher's exact test). Since this association has not been reported before we analysed the clinical records of different studies and case reports on CGCG[1–3,15] (Supplementary Table 1, Appendix A). This analysis confirms that the correlation between age and location of the Italian cohort is not a common feature of CGCG (Supplementary Fig. 1, Appendix A). Future studies will disclose whether *FGFR1* mutations, young age and CGCG location are interconnected.

The small size of our cohort might provide a clue on this aspect. The discrepancies between the Italian cohort and Gomes *et al.*, in terms of both *FGFR1* mutation frequency in young CGCG patients and *FGFR1* mutation pattern (with the majority of Italian CGCG cases harbouring pN330I mutation and the majority of Gomes *et al.* harbouring pC381R mutation) could be due to a number of factors. Different genetic predisposition to *FGFR1*-mutated CGCG in young patients that differ by ethnic group and/or different environmental influences could be involved. Further studies might identify germline mutations in predisposing genes in young patients with *FGFR1*-mutant CGCG. In our cohort, patients shared both geographical and ethnic features (Italian, Caucasian patients). No information is publicly available for the Gomes *et al.* cohort, though a certain grade of heterogeneity could be hypothesised.

Different *FGFR1* mutation frequency, according to cohort composition, has been observed before. Sun *et al.* reported a higher *FGFR* aberration frequency in Chinese colorectal and breast cancer patients compared to Caucasian cohorts.[16] Welander *et al.* described a prevalence of *FGFR1* mutations of almost 10% in sporadic phaeochromocytomas of a Scandinavian cohort, while no *FGFR1* mutations were identified in the French validation set of the same study.[17]

In conclusion, our analysis highlighted a high frequency of *FGFR1* mutations in CGCG from young patients (<18 years of age at diagnosis). Our observation might help prioritise candidates for *FGFR1* testing, thus opening treatment options with FGFR inhibitors for young CGCG patients. These drugs are currently being tested in different tumour types[18] and could be used for the treatment of difficult CGCG cases harbouring *FGFR1* gain of function mutations. Further investigation in larger cohorts is necessary to confirm the associations between *FGFR1* mutations, tumour location, young age, genetic, ethnic and/or environmental features in CGCG patients. Moreover, monitoring of patients will be required to assess a possible correlation between *FGFR1* mutations and recurrence.

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Data availability: WES data were deposited to the Sequence Read Archive (accession number: PRJNA861134). All relevant data are available from the authors upon reasonable request.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2022.09.003.

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References

1. Schreuder WH, Peacock ZS, Ebb D, Chuang SK, Kaban LB. Adjuvant antiangiogenic treatment for aggressive giant cell lesions of the jaw: a 20-year experience at Massachusetts General Hospital. *J Oral Maxillofac Surg* 2017; 75: 105–18.
2. Schreuder WH, van den Berg H, Westermann AM, Peacock ZS, de Lange J. Pharmacological and surgical therapy for the central giant cell granuloma: a long-term retrospective cohort study. *J Craniomaxillofac Surg* 2017; 45: 232–43.
3. Gomes CC, Gayden T, Bajic A, *et al*. TRPV4 and KRAS and FGFR1 gain-of-function mutations drive giant cell lesions of the jaw. *Nat Commun* 2018; 9: 4572.
4. The Usadel Lab. Trimmomatic: a flexible read trimming tool for Illumina NGS data. Cited Jun 2022. http://www.usadellab.org/cms/?page=trimmomatic#.
5. Sentieon. Software Manual for Sentieon® Genomics pipeline tools. Cited Jun 2022. https://support.sentieon.com/versions/202112/manual/.
6. GitHub. samtools/bcftools. Cited Jun 2022. https://github.com/samtools/bcftools/releases/tag/1.16.
7. McLaren W, Gil L, Hunt SE, *et al*. The Ensembl variant effect predictor. *Genome Biol* 2016; 17: 122.
8. National Cancer Institute. GDC MAF Format. Cited Jun 2022. https://docs.gdc.cancer.gov/Data/File_Formats/MAF_Format/.
9. SIFT. Sorting Tolerant From Intolerant. Cited Jun 2022. https://sift.bii.a-star.edu.sg/.
10. PolyPhen-2 prediction of functional effects of human nsSNPs http://genetics.bwh.harvard.edu/pph2/.
11. Robinson JT, Thorvaldsdóttir H, Winckler W, *et al*. Integrative genomics viewer. *Nat Biotechnol* 2011; 29: 24–6.
12. Ma X, Liu Y, Alexandrov LB, *et al*. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature* 2018; 555: 371–6.
13. Lee W, Wang Z, Saffern M, Jun T, Huang KL. Genomic and molecular features distinguish young adult cancer from later-onset cancer. *Cell Rep* 2021; 37: 110005.
14. de Lange J, van den Akker HP, Klip H. Incidence and disease-free survival after surgical therapy of central giant cell granulomas of the jaw in The Netherlands: 1990-1995. *Head Neck* 2004; 26: 792–5.
15. Triantafillidou K, Venetis G, Karakinaris G, Iordanidis F. Central giant cell granuloma of the jaws: a clinical study of 17 cases and a review of the literature. *Ann Otol Rhinol Laryngol* 2011; 120: 167–74.
16. Sun Y, Li G, Zhu W, *et al*. A comprehensive pan-cancer study of fibroblast growth factor receptor aberrations in Chinese cancer patients. *Ann Transl Med* 2020; 8: 1290.
17. Welander J, Łysiak M, Brauckhoff M, *et al*. Activating FGFR1 mutations in sporadic pheochromocytomas. *World J Surg* 2018; 42: 482–9.
18. Facchinetti F, Hollebecque A, Bahleda R, *et al*. Facts and new hopes on selective FGFR inhibitors in solid tumors. *Clin Cancer Res* 2020; 26: 764–74.

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