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CASE REPORT

TNFRSF13B gene mutation in familial acute myeloid leukemia: A new piece in the complex scenario of hereditary predisposition?

Cosimo Cumbo[1] | Paola Orsini[2] | Francesco Tarantini[1] | Luisa Anelli[1] | Antonella Zagaria[1] | Vincenzo Tragni[3] | Nicoletta Coccaro[1] | Giuseppina Tota[1] | Elisa Parciante[1] | Maria Rosa Conserva[1] | Immacolata Redavid[1] | Crescenzio Francesco Minervini[1] | Angela Minervini[1] | Immacolata Attolico[1] | Mattia Gentile[2] | Ciro Leonardo Pierri[3] | Giorgina Specchia[4] | Pellegrino Musto[1] | Francesco Albano[1]

[1]Hematology and Stem Cell Transplantation Unit, Department of Precision and Regenerative Medicine and Ionian Area (DiMePRe-J), University of Bari "Aldo Moro", Bari, Italy

[2]Medical Genetics Unit, Department of Human Reproductive Medicine, ASL Bari, Bari, Italy

[3]Laboratory of Biochemistry, Molecular and Computational Biology, Department of Pharmacy – Pharmaceutical Sciences, University of Bari, Bari, Italy

[4]School of Medicine, University of Bari "Aldo Moro", Bari, Italy

Correspondence
Francesco Albano, Hematology and Stem Cell Transplantation Unit, Department of Precision and Regenerative Medicine and Ionian Area (DiMePRe-J), University of Bari "Aldo Moro", Bari, Italy.
Email: francesco.albano@uniba.it

Abstract

TNFRSF13B mutations are widely associated with common variable immunodeficiency. *TNFRSF13B* was recently counted among relevant genes associated with childhood-onset of hematological malignancies; nonetheless, its role in acute myeloid leukemia (AML) remains unexplored. We report the study of a family with two cases of AML, sharing a germline *TNFRSF13B* mutation favoring the formation of a more stable complex with its ligand TNFSF13: a positive regulator of AML-initiating cells. Our data turn the spotlight onto the *TNFRSF13B* role in AML onset, inserting a new fragment into the complex scenario of a hereditary predisposition to myeloid neoplasms.

KEYWORDS
familial AML, hereditary predisposition, *TNFRSF13B*

Cosimo Cumbo, Paola Orsini, and Francesco Tarantini contributed equally to this work.

1 | INTRODUCTION

Tumor necrosis factor (TNF)-like receptors are members of a superfamily of genes that transduce key signals to regulate both the survival and apoptosis of immune cells. The *TNF receptor superfamily member 13 b* (*TNFRSF13B*) gene encodes TACI, a lymphocyte-specific member of the TNF receptor superfamily, and its mutations are widely associated with common variable immunodeficiency (CVID).[1] Although CVID patients have an overall increased risk of hematological malignancies,[2] few data are available on *TNFRSF13B* role in leukemogenesis. In a recent nationwide study of cancer-prone syndromes (analyzing 198 pediatric cancer patients), *TNFRSF13B* was counted among relevant genes associated with childhood-onset of hematological malignancies.[3] Furthermore, common variants of *TNFRSF13B* and *TNFSF13* (encoding APRIL, the main TACI-ligand) were recently associated with the chronic lymphocytic leukemia (CLL) risk, as well as the expression of their products (TACI and APRIL).[4] Nonetheless, the involvement of constitutive *TNFRSF13B* alterations in acute myeloid leukemia (AML) remains unexplored. Herein we report the study of a family with two cases of AML, sharing a germline *TNFRSF13B* mutation (Figure 1A), with a candidate role in predisposing to familial AML onset.

A 74-year-old Caucasian male was referred to our center for pancytopenia (WBC 1.1 × 10^9/L, Hb 6.7 g/dL, PLT 67 × 10^9/L); a peripheral blood smear showed the presence of myeloid blasts (>20%). At the end of the hematological work-up, the patient was diagnosed with AML without maturation.[5] The karyotype was normal. He underwent three cycles of azacitidine and eventually died due to uncontrolled sepsis. Of note, during the collection of his past medical history, the patient referred that he had two monozygotic twin sons, one of whom had been diagnosed with AML 23 years before, when he was a 16-year-old, and treated at our center. This latter was successfully cured with a syngeneic donor hematopoietic stem cell transplant (HSCT), and nowadays, he is alive and in good clinical conditions. Notably, none of our probands had an immunological compromission in their past medical history. These data suggested a deeper study of the biological aspects of the disease to investigate the possible occurrence of germline variants predisposing to AML onset. The study was approved by the local ethics committee of "Azienda Ospedaliero Universitaria Policlinico di Bari." Written, informed consent was obtained from the patients before study inclusion, in accordance with the Declaration of Helsinki. Patients' records/information were anonymized and de-identified before analysis. Written consent for publication was obtained before their enrollment in the present study.

Targeted next-generation sequencing analysis with a customized panel encompassing 26 genes involved in the pathogenesis of myeloid malignancies was performed on bone marrow (BM) samples of both AML patients (father and son) at the time of diagnosis, as previously reported (Figure 1B, cases I:1 and II:1) (Supporting Information S1).[6] No constitutive variants affecting the known genes associated with familial AML[7] were identified. The probands showed two different mutational profiles: case I:1 showed a missense variant in *TP53* (NM_000546) c.722C > A, p.Ser241Tyr [variant allele frequency (VAF): 76.7%], while case II:1 showed a frameshift insertion in *NPM1* (NM_002520) c.863_864insCCTG, p.Trp288CysfsTer12 (VAF: 51.5%) and a missense variant in *IDH2* (NM_002168): c.419G > A, p.Arg140Gln (VAF: 52.3%).

Whole exome sequencing (WES) was performed on the same samples (Supporting Information S1). The mean depth of the two sequencing runs was 139.4X and 111.7X, respectively. Overall, 23,974 and 24,133 variants were called in the two samples. The files of annotated variants obtained were filtered as described in Supporting Information S1; overall, 21 final variants shared between the two samples were retained (Supporting Information S3: Table S1). No variants in genes associated with myeloid neoplasms were identified. However, the two samples shared a missense variant in *TNFRSF13B* (NM_012452.2): c.310T > C, p.Cys104Arg (rs34557412, COSMIC ID: COSM3766009, Clinvar Variation ID: 5302), with a VAF of 65% and 48.3% in case I:1 and case II:1, respectively (Figure 1A,B). Notably, the variant was previously described as pathogenic in a functional study in association with CVID.[8]

The germline origin of the *TNFRSF13B* variant was verified by direct sequencing (Figure 1C) on genomic DNA extracted from the buccal swab of two patients (cases I:1 and II:1) and donor (case II:2) (Supporting Information S1).

A 3D modeling analysis (Figure 1D–F) was performed to predict the effect of the *TNFRSF13B* mutational status on TACI activity (Supporting Information S1). The human TACI crystallized structure in complex with its ligand APRIL was identified along pGenThreader and I-tasser searches as the ideal protein template for building the 3D comparative model of TNFRSF13B_C104R mutant. The replacement of C104 with an arginine residue destabilizes one (the second) of the two cysteine-rich domains (CRD), typically present in most TNF receptors. The C104 replacement causes loss of the disulfide bridge (with specific reference to the disulfide bridge between C104-C93, see Figure 2A) at the second CRD, destabilizing the structure of TNFRSF13B subunit, and perturbing local secondary structure elements, and the disulfide bridge network. This latter is crucial for stabilizing the TNF homology domain, which is a common structural domain shared by TNF ligands, such as the proliferation-inducing ligand (APRIL) and B cell activating factor.[9] The replacement of C104 with an arginine residue favors the formation of new hydrophilic and ionic interactions (i.e., between R104 and E105, see Figure 2B), due to the greater mobility acquired by TNFRSF13B_C104R, eased by the loss of a disulfide bridge on each monomer and due to the more hindering and charged arginine side-chain. A greater flexibility of TNFRSF13B causes higher stability in the complex with its ligand; in fact, interaction energies calculated between TNFRSF13B_C104R and the TNFSF13 ligand are stronger (more negative values, i.e., −68.92 kcal/mol) than interaction energies calculated between the wild-type TNFRS13B and its ligand (−64.87) (Supporting Information S4: Table S2).

Of note, none of our probands showed a CVID phenotype; in fact, gene dosage, mutation type and additional clinical or laboratory abnormalities influence the relevance of *TNFRSF13B* variants in CVID

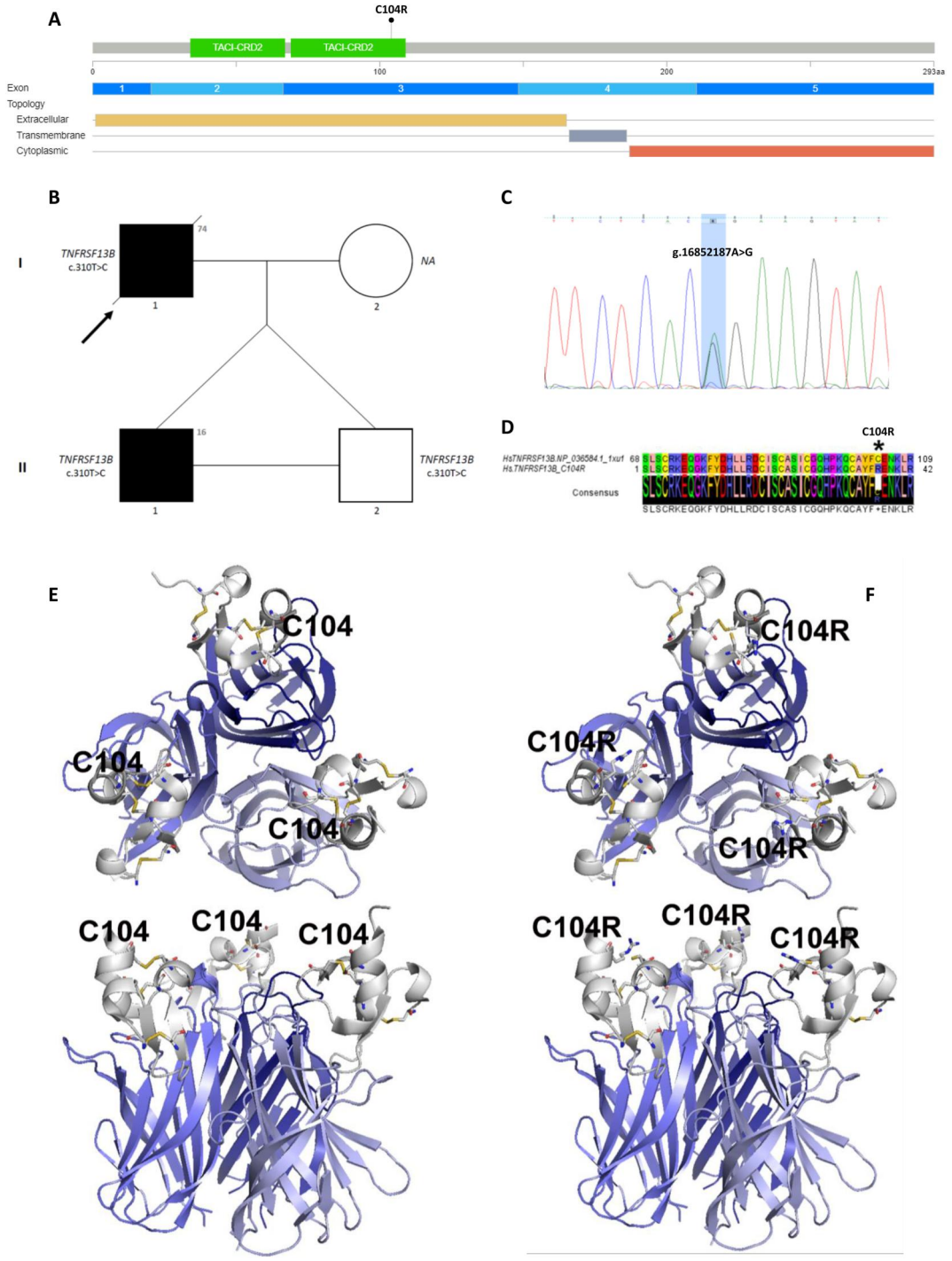

FIGURE 1 Molecular characterization and 3D modeling of *TNFRSF13B* C104R variant. (A) Map of C104R mutation identified on a linear schematization of the TNFRSF13B protein (lollipop plots). The g.16852187A > G variant (exon 3) causes the replacement of C104 with an arginine residue, destabilizing the second of the two cysteine-rich domains (CRD) in the extracellular subunit of the protein. (B) Pedigree representation of the described family. The *TNFRSF13B* variant (c.310T > C; p.C104R) was identified in the two patients (I:1 and II:1) and the healthy donor (II:2). Age of patients is indicated as numbers in gray font color. The index case is indicated with an arrow. NA: not available (C) Direct sequencing of genomic DNA extracted from the buccal swab, confirming the germline origin of the g.16852187A > G variant. (D) Sequence Alignment (MSA) of the human TNFRSF13B amino acid sequence residues 68–109 according to the NP_0036584.1 sequence numbering, deposited on the refseq_protein database, and the corresponding amino acid sequence residues 1–42 according to the crystallized structure "1xu1.pdb" sequence numbering, in which the investigated amino acid replacement C104R is reported. The "*" symbol indicates the C104R amino acid replacement position. (E,F) The top view (upper portion of panels E,F) and side view (bottom portions of panels E,F) of the *H. sapiens* TNFRSF13B receptor and TNFRSF13B_C104R mutant receptor, reported in the gray cartoon representation, are shown in complex with TNFSF13 ligand (according to 1xu1.pdb), represented in the light blue cartoon representation. Cysteine residues are reported in white/yellow sticks and labeled. The C104R amino acid replacement is shown as white/blue sticks and labeled in panel F.

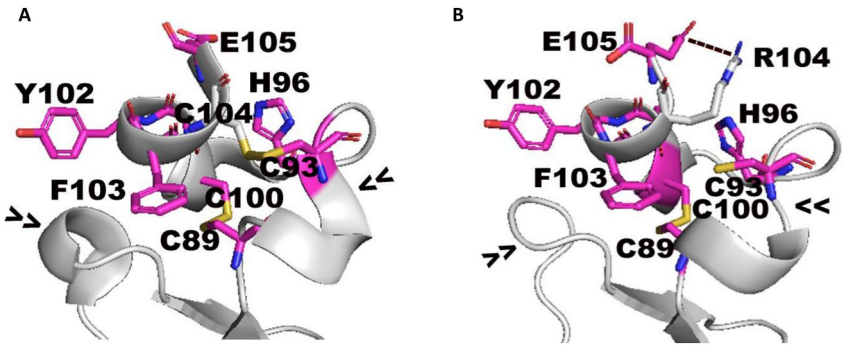

FIGURE 2 Zoomed views of TNFRSF13B and TNFRSF13B_C104R reported in white cartoon representation. C104 and R104 are shown as white sticks. Residues within 4 Å from C104 (A) or R104 (B) are shown as magenta sticks. ">>" and "<<" highlight a perturbation in the indicated local secondary structure elements due to the replacement of C104 with an arginine residue. The dashed line indicates a possible newly established ionic interaction between R104 and E105 in the C104R mutant (B), following the loss of the C104-C93 disulfide bridge (A).

phenotype, as demonstrated in several studies of TACI-deficient humans and murine models, revealing novel aspects of TACI cross-talk with the Toll-like receptors pathways, differential expression of TACI isoforms, and its role in the generation of autoreactive B-cells.[1]

The 5th edition of the World Health Organization (WHO) Classification of Hematolymphoid Tumors has better defined the subtypes of myeloid neoplasms associated with germline predisposition, suggesting a formulaic approach that couples the myeloid disease phenotype with the predisposing germline genotype.[10] New gene mutations have been reported as compared to the previous WHO classification, and genetic counseling and evaluation of family history are now considered an important component of the diagnostic process of index patients.[10]

Our study elects TNFRSF13B as a new candidate gene predisposing to AML onset. The germline variant (C104R) identified in our family allows a greater flexibility of TNFRSF13B, most likely favoring the formation of a more stable complex with its ligand TNFSF13. This latter is a positive regulator of AML-initiating cells; it promotes AML initiation by suppressing apoptosis and activating cell cycle progression.[11] Of note, TNFSF13 supports AML initiation and maintenance also under physiological conditions, being an optimal candidate in predisposing to AML onset. In fact, in mice, TNFSF13 is secreted by normal myeloid but not by leukemia cells, suggesting that mature myeloid BM cells support leukemia cells by secreting TNFSF13.[11] The higher stability of the ligand-receptor complex could physiologically produce a proliferative stimulus, favoring leukemogenesis, similarly to the phenomenon observed in the case of overexpression of TNFSF13, which may cause a higher susceptibility to CLL due to the saturation (in terms of a more significant number of interactions) of TNFRSF13B.[4] Larger data support our observations; in fact, in a recent nationwide study on cancer-prone syndromes, *TNFRSF13B* was reported among relevant genes associated with the childhood onset of glioma, Burkitt lymphoma and Langerhans cell histiocytosis.[3] Furthermore, as stated above, common *TNFRSF13B* variants and TACI expression were recently correlated with the risk of CLL onset.[4]

Last but not least, the family presented in this study offers an important point of reflection on the appropriate weight that should be attributed to "hereditary predispositions."[12] The latest ELN recommendations on adult AML diagnosis and management highlight the relevance of recognizing hereditary predispositions, especially if there is any question of implementing allo-HSCT and health surveillance strategies for the patient and relatives who share the causative variant.[13] Notably, in our family, case II:2, despite presenting the same genotype as his affected twin (case II:1), is alive and in good clinical conditions. Twenty-three years before, when case II:1 was the only affected member in the family, the data from WES were not available, and there was no suspicious of a germline predisposition for a familial disease; he had been the graft source for the syngeneic HSCT that cured his brother.

In this context, what is the best weight to attribute to germline genomic aberrations, and how much impact should they have on clinical practice? Considering the limitations associated with the retrospective nature of our study, further data on a more significant number of cases are warranted to clarify this critical aspect of patient management.

In conclusion, our data turn the spotlight onto the *TNFRSF13B* role in AML onset, inserting a new fragment into the complex scenario of a hereditary predisposition to myeloid neoplasms and highlighting the ever-central role of acquired events in the disease onset, also in the fertile ground of genetic susceptibility.

AUTHOR CONTRIBUTIONS

Conception and design of the study: Cosimo Cumbo and Francesco Albano. *Whole exome sequencing*: Paola Orsini and Mattia Gentile. *3D modeling analysis*: Vincenzo Tragni and Ciro Leonardo Pierri. *Acquisition of data and/or analysis and interpretation of data*: Cosimo Cumbo, Francesco Tarantini, Luisa Anelli, Antonella Zagaria, Nicoletta Coccaro, Giuseppina Tota, Elisa Parciante, Maria Rosa Conserva, Immacolata Redavid, Crescenzio Francesco Minervini, Angela Minervini, Giorgina Specchia, Pellegrino Musto and Francesco Albano. *Clinical data acquisition*: Francesco Tarantini and Immacolata Attolico.

Drafting of the manuscript: Francesco Albano. All authors revised the manuscript for important intellectual content and approved the final version submitted for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files.

ORCID

Cosimo Cumbo https://orcid.org/0000-0002-2509-6200
Francesco Albano https://orcid.org/0000-0001-7926-6052

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SUPPORTING INFORMATION

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