

Scientific Letter

The Possible Role of Chronic Infection in the Etiopathogenesis of a Case of 5q-Syndrome Associated with Tuberculosis and Abnormality of the X Chromosome

Keywords: 5q-Syndrome; Tuberculosis; Abnormality of the X chromosome; WWC1 (Kibra).

Published: November 1, 2022

Received: September 8, 2022

Accepted: October 16, 2022

Citation: Mitev L., Grahlyova L., Kancheva T. The possible role of chronic infection in the etiopathogenesis of a case of 5q-syndrome associated with tuberculosis and abnormality of the X chromosome. *Mediterr J Hematol Infect Dis* 2022, 14(1): e2022079, DOI: <http://dx.doi.org/10.4084/MJHD.2022.079>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

To the editor.

We read with great interest the excellent papers by Pellagatti &, Boulwood, and Leone & Pagano recently published in the *Mediterranean Journal of Hematology and Infectious Diseases*.^{1,2} Both paper concern Myelodysplastic Syndromes. The first highlights the importance of the genes mapped to the commonly deleted region, such as RPS14, miR-145, miR-146a, and CSNK1A1, for the pathogenesis of 5q- syndrome; the second claims that bacterial infections are a secondary event mainly due to defects in the myeloid lineage and to myelosuppressive therapy. Here we describe a case of 5q-syndrome associated with tuberculosis and a new clonal abnormality - a translocation between chromosome X and a chromosome 5 deleted in its long arm. We identified that near or at the breakpoints of the rearrangement are located the genes ARRDC3, RAB5CP2, WWC1, ENSG00000237311, and miR-223. Based on their functions, we hypothesize that, in our

case, the bacterial infection could also be a primary event causing the occurrence of clonal hematopoiesis.

A 73-year-old woman was referred to the clinic with complaints of easy fatigue and a history of pneumonia from the previous year. Laboratory tests showed hemoglobin 90g/l, mean corpuscular volume 98fl, erythrocyte sedimentation rate (ESR) 23mm, white blood cells (WBC) 4.5x10⁹/l (neutrophils 3.51x10⁹/l) and platelets 87x10⁹/l. The X-ray found fibrocalcific changes in both hilus of the lung. The bone marrow examination showed normocellular marrow with 1% blasts, megaloblastic erythropoiesis, hypo- or agranular mature neutrophils (22%), and an increased number of megakaryocytes (1%) with prominent dysplasia (38% hypo- or non-lobated micromegakaryocytes). Conventional cytogenetics revealed the following new clonal chromosome rearrangement: 46,X,t(X;5)(Xpter=>Xq12::5q34=>5qter;5pter>5q14::Xq12=>Xqter)[20]/46,XX[5] (Figure 1).

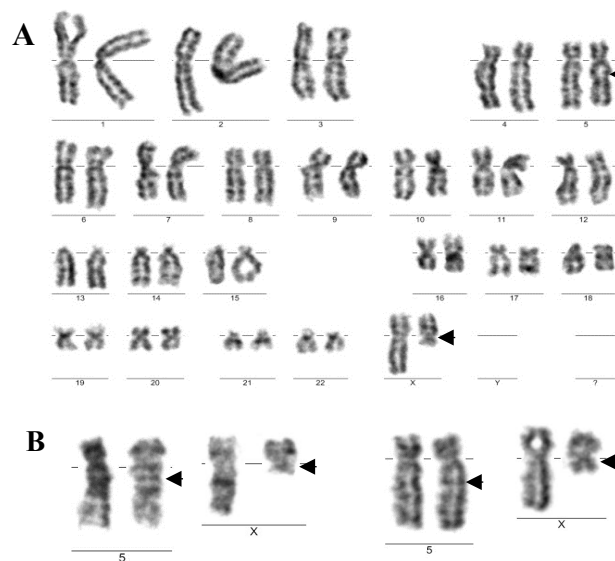


Figure 1. Conventional cytogenetics. A) G-banded karyotype showing 46,X,der(5)t(X;5)(q12;q34),del(5) (q14q34). B) G-banded partial karyotypes of chromosome X and 5 homologues from two metaphases showing der(5)t(X;5)(q12;q34)del(5)(q14q34). The breakpoints are arrowed.

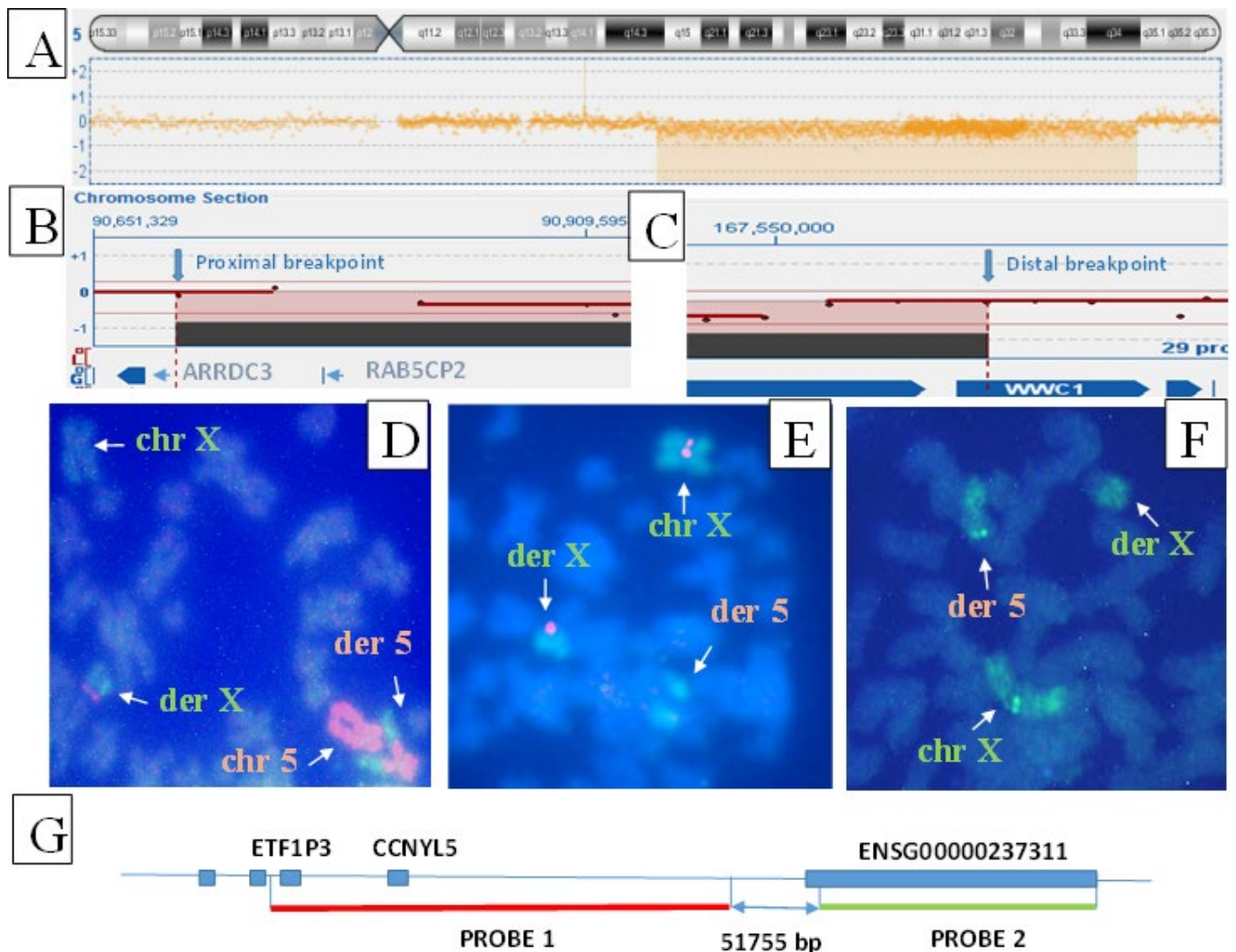


Figure 2. Molecular cytogenetics **A)** Ideogram and microarray CGH plot of chromosome 5 showing a gain in the band 5q14.1 and interstitial 5q deletion del(5)(q14.3q34). **B)** Microarray CGH plot showing the location of the breakpoint 5q14.3 between the genes ARRDC3 and RAB5CP2 (arrowed). **C)** Microarray CGH plot showing the location of the breakpoint 5q34 within the gene WWC1 (arrowed). **D)** Fluorescence in situ hybridization of chromosome X (green) and 5 (red) with whole chromosome probes demonstrating t(X;5). **E)** Fluorescence in situ hybridization of chromosome X with whole chromosome probe (green) and locus-specific probe covered the chromosome region Xq12:65,770,170-65,874,155 (red). **F)** Fluorescence in situ hybridization of chromosome X with whole chromosome probe (green) and a locus-specific probe for the gene ENSG00000237311 (green). **G)** The DNA region where the breakpoint Xq12 is occurred (between probe 1 and probe 2).

No stem line with del(5)(q14q34) was detected, which suggests that 5q- and t(X;5) have arisen simultaneously. Fluorescence in situ hybridization (FISH) with whole chromosome probes for X and 5 confirmed t(X;5):46, X ish t(X;5)(q12;q34)(wcpX+,wcp5+;wcp5+.wcpX+) (Figure 2D). Microarray comparative genomic hybridization with 60K microarray platform (OGT, Oxford, UK) established two unbalanced anomalies in 5q: gain at band 5q14.1 and loss at 5q14.3 through 5q34 which are with 367.91Kb and 77.05Mb in size: arr[hg19] 5q14.1(79,161,513-79,529,419)x3,5q14.3q34(90,695,096-167,742,618)x1 (Figure 2A). The breakpoint in 5q14.3 occurred in the noncoding region between the genes ARRDC3 and

RAB5CP2 (Figure 2B) and in 5q34 within the WWC1 gene (Figure 2C) (validated by FISH). To determine the breakpoint location on Xq12 FISH experiments were performed with whole staining of the X chromosome combined with two locus-specific probes - one covered the chromosome region Xq12:65,770,170-65,874,155 (104kb; hg38) and another the gene ENSG00000237311 (Xq12:65,925,930-66,001,435;hg38) (Agilent Technologies, Santa Clara, CA, USA). The signal from the first probe was found on the X chromosome (Figure 2E), while the signal from the second probe was on chromosome 5 (Figure 2F). These data revealed that the breakpoint on Xq12 occurred in the 52kb region (56,925,930-66,001,435) (hg38) that included 47.5kb noncoding DNA sequences and 4.5 kb coding sequences

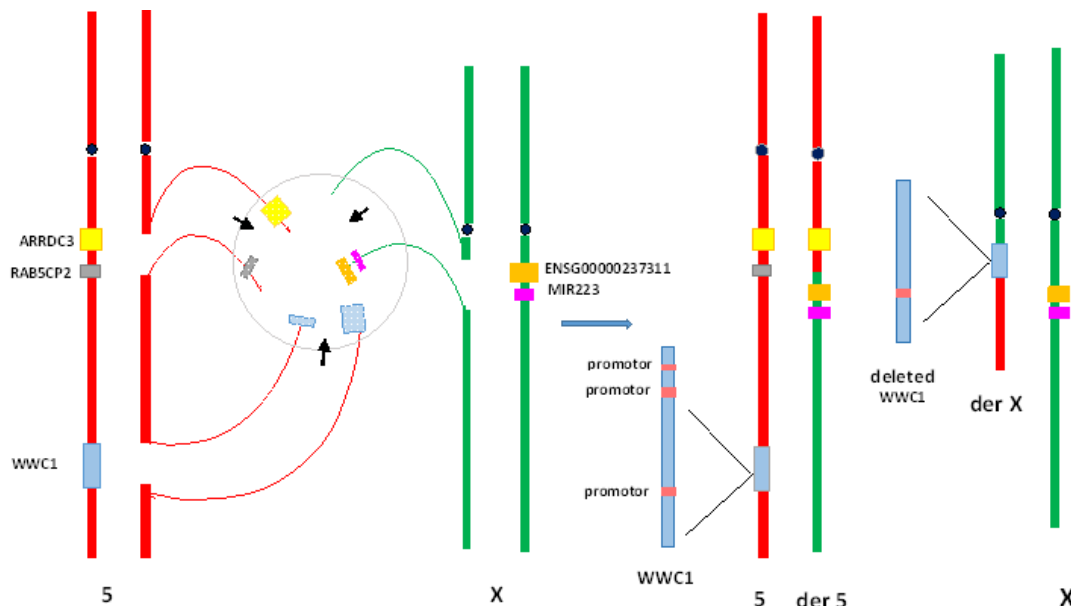


Figure 3. Principle scheme of the formation of der(5)t(X;5(q12;q34), del(5)(q14q34) and its cytogenetic and molecular consequences.

from the 5' end of the ENSG00000237311 gene (**Figure 2G**). Collectively, the genomic studies showed that 1) del(5)(q14.3q34) resulted in a loss of the whole RAB5CP2 gene and part of the WWC1 gene (the size of the lost part is 23862bp and included the enhancer, the WW domains, and the first and second promoter) and 2) t(X;5) caused juxtaposition of the deleted WWC1 gene to the X chromosome, and ENSG00000237311 gene to chromosome 5 respectively (near to ARRDC3 gene) (**Figure 3**). The diagnosis of 5q- syndrome was made, and therapy with lenalidomide was started at a reduced dose of 5 mg daily because of thrombocytopenia. Over the next two months, along with pancytopenia caused by the lenalidomide, a gradual increase of monocytes, ESR, and C-reactive protein (CRP) was observed. At the end of the third month, the patient was re-admitted to the clinic with a deteriorating general condition, low-grade fever (37.2°C), pancytopenia (hemoglobin 70g/l, WBC $2.1 \times 10^9/l$, and platelets $52 \times 10^9/l$), neutropenia ($0.39 \times 10^9/l$), relative monocytosis (33%, $0.7 \times 10^9/l$) and increased inflammation markers (ESR 70 mm and CRP 17.8 mg/l). Body examination found two enlarged cervical lymph nodes (2.5 cm in diameter) in the supraclavicular area, which were movable, painless, and firm in consistency. Histopathology after excisional lymph node biopsy revealed granulomatous lymphadenitis with epithelioid and giant cells. QuantiFERON-TB gold test was positive and confirmed the diagnosis of extrapulmonary TB manifested as tuberculosis lymphadenitis. The clinical condition was considered a reactivation of latent TB into active TB caused by lenalidomide treatment.² The therapy with lenalidomide was discontinued, and an anti-TB medication with ethambutol 15 mg/kg, isoniazid 300 mg/day, rifampicin 600 mg/day, and pyrazinamide 15 mg/kg was started. After six months of anti-TB therapy, the patient's lymphadenopathy disappeared, and the

number of neutrophils and platelets increased to $1.7 \times 10^9/l$ and $80 \times 10^9/l$, respectively. Monocytes, ESR, and CRP, decreased to normal values. Lenalidomide treatment was re-initiated at a reduced dose of 5 mg every other day. During the next four years, the patient maintained transfusion independence (hemoglobin up to 100 g/l) and good quality of life.

The functions of the described genes will clarify the cellular events that have provoked der(5)t(X;5), del(5q). ARRDC3 is a member of the alpha arrestins that mediate endosome trafficking leading to recycling or lysosomal degradation of G protein-coupled receptors.³ The second gene, RAB5CP2, is a pseudogene. Its possible function as a pseudogene is to regulate the expression of its parent gene⁴ RAB5C, which is a regulator of the early stage of the endocytic pathway⁵. The protein of the third gene WWC1 (Kibra) (5q34) as part of the protein complexes dynein/SNX4 and exocyst/PKCzeta mediate the direction of transferrin receptor to a recycling pathway and transcriptional co-activation of estrogen receptor – alpha^{6,7} respectively. Kibra is also involved in the regulation of autophagosomes, promoting autophagosomal degradation via the Hippo pathway (through its WW domains).⁸ The data from these three genes suggest an obvious regularity - the proteins encoded by them are related to the endocytic and phagosomal pathways that are closely interconnected and are involved in cellular homeostasis, including the protection of organisms from invading pathogens. It is also interesting that the located at Xq12 gene ENSG00000237311 is a long noncoding RNA with unknown function, but close to it is mapped miR-223, whose increased expression has been found in both 5q-syndrome and tuberculosis.^{9,10} Moreover, miR-223 is critical in mediating the innate host response to tuberculosis.¹¹ Finally, it is logical to suppose that, in our case, the loss of RAB5CP2 from the genome and the

deletion of WW domains of WWC1 can affect the maturation of the early Rab 5 phagosomes and the hypo pathway with subsequent blocking of the lysosomal degradation of the pathogen. The consequences of the described anomaly could favor the cellular adaptation of the bacterial pathogen, consistent with the reports demonstrating that *Mycobacterium tuberculosis* can manipulate early Rab 5 phagosomes and the Hippo pathway to modulate the host immune response.^{10,12}

In conclusion, the appearance of the established clonal abnormality in our case is probably linked to the signaling network induced by the bacterial pathogen to avoid the host's innate immune system, which possibly includes also sex-dependent mechanisms such as deregulation of noncoding RNA genes located on the X chromosome.

Lubomir Mitev¹, Liliya Grahlyova¹ and Tatiana Kancheva².

¹ Department of Cytogenetics and Molecular Biology, Military Medical Academy, Sofia, Bulgaria.

² Clinic of Hematology, Military Medical Academy, Sofia, Bulgaria.

Competing interests: The authors declare no conflict of Interest.

Correspondence to: Lubomir Mitev. E-mail: cytogen@vma.bg

References:

1. Pellagatti A, Boultonwood J. Recent Advances in the 5q- Syndrome. *Mediterr J Hematol Infect Dis*. 2015 May 20;7(1):e2015037. <https://doi.org/10.4084/mjihid.2015.037> PMID:26075044 PMCID:PMC4450650
2. Leone, G. and Pagano, L. (2018) Infections in myelodysplastic syndrome in relation to stage and therapy, *Mediterranean Journal of Hematology and Infectious Diseases*, 10(1), p e2018039. <https://doi.org/10.4084/mjihid.2018.039> PMID:30002795 PMCID:PMC6039080
3. Puca L, Brou C. α -Arrestins - new players in Notch and GPCR signaling pathways in mammals. *J Cell Sci*. 2014; 127 (7):1359-1367. <https://doi.org/10.1242/jcs.142539> PMID:24687185
4. Yang An, Kendra LF, Shaoping Ji. Pseudogenes regulate parental gene expression via ceRNA network. *J Cell Mol Med*. 2017; 21(1):185-192. <https://doi.org/10.1111/jcmm.12952> PMID:27561207 PMCID:PMC5192809
5. Zeigerer A, Gilleron J, Bogorad RL, Marsico G, Nonaka H, Seifert S, Epstein-Barash H, Kuchimanchi S, Peng CG, Ruda VM, Del Conte-Zerial P, Hengstler JG, Kalaidzidis Y, Koteliensky V, Zerial M. Rab5 is necessary for the biogenesis of the endolysosomal system in vivo. *Nature*. 2012 May 23;485(7399):465-70. <https://doi.org/10.1038/nature11133> PMID:22622570
6. Traer CJ, Rutherford AC, Palmer KJ, Wassmer T, Oakley J, Attar N, Carlton JG, Kremerskothen J, Stephens DJ, Cullen PJ. SNX4 coordinates endosomal sorting of TfnR with dynein-mediated transport into the endocytic recycling compartment. *Nat Cell Biol*. 2007 Dec;9(12):1370-80. <https://doi.org/10.1038/ncb1656> PMID:17994011
7. Rayala SK, den Hollander P, Manavathi B, Talukder AH, Song C, Peng S, Bamekow A, Kremerskothen J, Kumar R. Essential role of KIBRA in co-activator function of dynein light chain 1 in mammalian cells. *J Biol Chem*. 2006 Jul 14;281(28):19092-9. doi: 10.1074/jbc.M600021200. Epub 2006 May 9. PMID: 16684779. <https://doi.org/10.1074/jbc.M600021200> PMID:16684779
8. Xiao L, Chen Y, Ji M, Dong J. KIBRA regulates Hippo signaling activity via interactions with large tumor suppressor kinases. *J Biol Chem*. 2011; 286(10):7788-7796. <https://doi.org/10.1074/jbc.M110.173468> PMID:21233212 PMCID:PMC3048666
9. Votavova H, Grmanova M, Merkerova D, Belickova M, Vasikova A, Neuwirtova R and Cermak J. Differential Expression of MicroRNAs in CD34+ Cells of 5q- Syndrome. *J Hematol Oncol*. 2011; 4, 1. <https://doi.org/10.1186/1756-8722-4-1> PMID:21211043 PMCID:PMC3024999
10. Brumell JH, Scidmore MA. Manipulation of rab GTPase function by intracellular bacterial pathogens. *Microbiol Mol Biol Rev*. 2007; 71(4):636-52. <https://doi.org/10.1128/MMBR.00023-07> PMID:18063721 PMCID:PMC2168649
11. Yuan S, Wu Q, Wang Z, Che Y, Zheng S, Chen Y, Zhong X, Shi F. miR-223: An Immune Regulator in Infectious Disorders. *Front Immunol*. 2021 Dec 10;12:781815. <https://doi.org/10.3389/fimmu.2021.781815> PMID:34956210 PMCID:PMC8702553
12. Boro M, Singh V, Balaji K. *Mycobacterium tuberculosis*-triggered Hippo pathway orchestrates CXCL1/2 expression to modulate host immune responses. *Sci. Rep*. 2016; 6, 37695; <https://doi.org/10.1038/srep37695> PMID:27883091 PMCID:PMC5121601