ANALYSIS OF HIV-1 INTEGRATION IN CELLS FROM PATIENTS AFFECTED BY A TNPO3-RELATED MUSCULAR DYSTROPHY

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BACKGROUND

TNPO3 is an importin involved in the transport of splicing factors such as CPSF6 to the nucleus. CPSF6 binds to the HIV-1 capsid and facilitates its translocation to the nucleus. Limb-girdle muscular dystrophy dominant 2 (LGMDD2) is a very rare autosomal dominant genetic muscle disease characterized by a heterozygous deletion in the TNPO3 gene, which generates a mutated isoform with 15 additional amino acids (Figure 1). Previous *in vitro* studies suggest that the cells of these patients are resistant to HIV-1 infection. The aim of this study is to characterize the integration sites (IS) of the virus in the genome of these patients' cells.

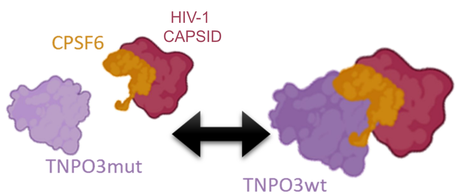


***Figure 1**. TNPO3 binds the splicing factor CPSF6, which in turn binds to the HIV-1 capsid (right). In LGMDD2 patients, a heterozygous deletion occurs in the TNPO3 gene, generating proteins with 15 extra amino acids that could alter the transport of CPSF6 to the nucleus (left).*

METHODS

PBMCs from 6 LGMDD2 patients and 5 unaffected relatives were isolated, activated with CD3+CD28+IL-2 for 72 hours, and infected with 50 ng p24/million cells of NL4.3-Renilla (Figure 2). At 24 hours, viral replication was assessed by measuring luciferase activity (Promega), and genomic DNA was isolated, fragmented, and specific ligands were added for sequencing (NEB-Next UltraII FS-DNA Library Prep Kit). Libraries were generated by PCR for the specific amplification of 3'LTR/5'LTR and genomic DNA hybrid fragments which were sequenced on MiSeq (Illumina).

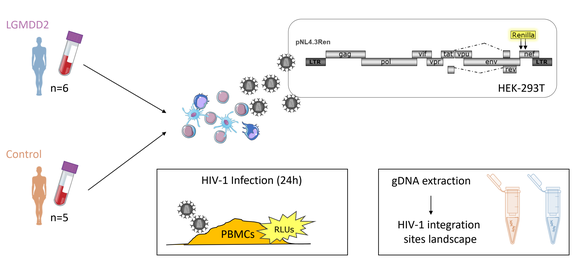


***Figure 2**. Methodology used for the characterization of integration sites of PBMCs infected in vitro with HIV-1.*

Resistance to HIV-1 infection observed in cells from patients with LGMDD2 may be related to the site in the human genome where the virus integrates during the first 24h after infection.

RESULTS

Luciferase activity was 1942±1435 in patients and 3189±1305 in controls (p=0.103, Figure 3). Library sequencing from 5 patients and 4 controls allowed the identification of an average of 21.6 and 24.8 IS respectively (p=0.6034, Figure 4A). In patients, 70.1% of IS were found in genes compared with 83.3% observed in controls (p=0.014, Figure 4B). Gene density of the regions in which the proviruses are integrated were slightly lower in patients (22.1 genes/Mb) compared with controls (25.7 genes/Mb; p=0.1247, Figure 5). Comparing the IS identified with data from ATAC-Seq experiments on activated CD4+ T lymphocytes (ENCODE Project), it was observed that proviruses integrated in genes located into accessible chromatin regions decreased to 46.1% in patients and to 64.4% in controls (p=0.031, Figure 6A). The proportion of IS located in the lamin-associated domains (LAD) of the nucleus in patients was 30.2% and 24.2% in controls (p=0.219, Figure 6B). No IS were located in centromeres or telomeres in any of the participants (Figure 5).

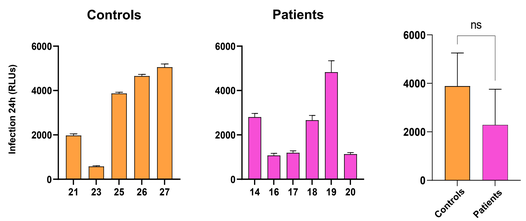


***Figure 3**. Relative light units (RLUs) obtained 24h after infection with NL4.3-Renilla in controls and patients and comparison between both groups showing mean and standard deviation.*

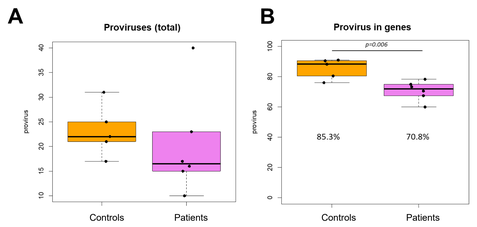


***Figure 4**. Characterization of the integration sites identified in patients and controls, including the total number of proviruses identified (A) and the percentage of proviruses identified in genes (B). Median and interquartile range are shown in all graphs, as well as the median value and the values for each participant.*

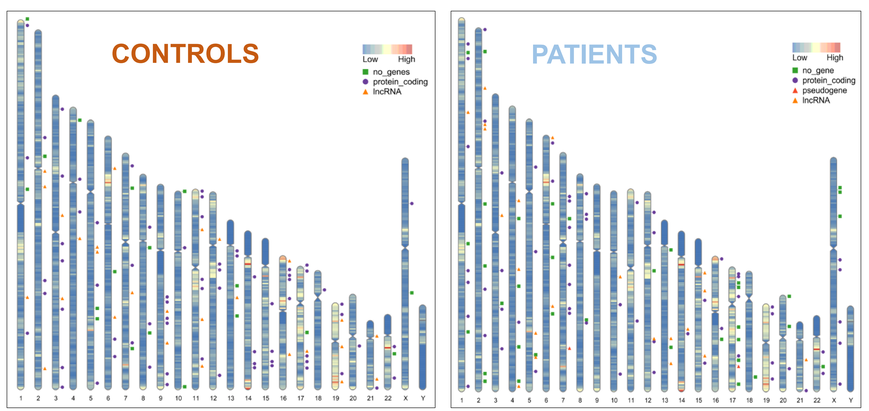


***Figure 5.** Schematic representation of the IS identified in patients and controls, indicating in each case whether the provirus has been integrated in an intergenic region (green squares), in genes encoding proteins (purple circle) or in genes encoding long non-coding RNAs (orange triangle). A representation of the gene density of each chromosomal region is also indicated according to the scale shown in the legend.*

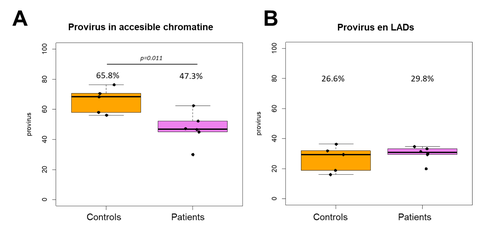


***Figure 6**. Characterization of the integration sites identified in patients and controls in accessible chromatin regions (A) and in nuclear lamina-associated domains (B). Median and interquartile range are shown in all graphs, as well as the median value and the values for each participant.*

CONCLUSIONS

The genetic mutation in TNPO3 observed in the cells of LGMDD2 patients appears to cause HIV-1 to decrease its preferential integration in genes, including those specifically located in accessible chromatin regions of activated CD4+ T lymphocytes, after 24 hours from infection, which may partly explain their resistance to infection.

ADDITIONAL KEY INFORMATION

Acknowledgements

Special gratitude to LGMDD2 patients for their commitment to research and for the gift of donated samples. The name of their Association is in Spanish, as the biggest part of the affected lives in Spain, but the meaning is this: “Conquering Steps”. The idea came from the fact that our life as affected of muscular dystrophy, among so many other things, is full of stairs: one of our first symptoms is, indeed, the difficulty to climb stairs. The course of the disease is characterized by a degenerative process which carries us to a wheelchair, and finally, when the internal organs are injured, to death. However, we have chosen the name “Conquistando Escalones” because the message that we want to spread is positive, as step by step, stair by stair, we will conquer the top of the mountain, finding the cure for our disease and for many other similar pathologies.

ASSOCIATION CONQUISTANDO ESCALONES

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PLAIN LANGUAGE SUMMARY

LGMDD2 patients express a mutated TNPO3 protein that is involved in the transport of HIV-1 to the nucleus and its integration into human chromosomes. This study demonstrates a decrease in HIV-1 integration into active gene regions when cells from these patients are infected.

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