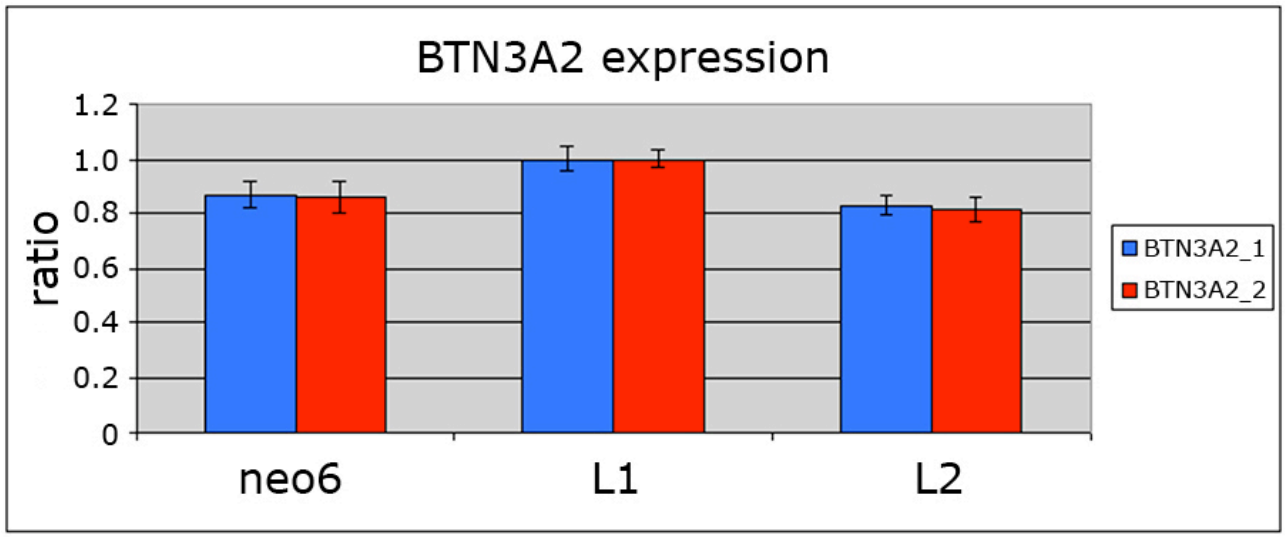
Supplemental Figure 2

BTN3A2 expression analysis



BTN3A2 expression was evaluated by reverse real time PCR using two different primer pairs (BTN3A2_1, in red, and BTN3A2_2, in blue; primer sequences are reported below). The analysis was performed on the lymphoblastoid cell line neo6, from the father of the proposita, and on two additional lymphoblastoid cell lines (L1 and L2) as control. The values are the average of three distint measurements. Total RNA was extracted from the lymphoblastoid cells using TriReagent (Sigma-Aldrich, St.Louis, USA). Total RNA was treated with DNase I (New England BioLabs Inc.) to remove possible genomic contamination and DNA-free RNA was retrotranscribed with SuperScript III (Invitrogen). Diluted RT reaction was used for Real Time PCR, using the IQ SYBR Green Supermix (Bio-Rad), performed on an IQ5 Real-time PCR machine (Bio-Rad). Primers for BTN3A2 were the following: AAGACAGCCAGCATTTCCAT (BTN3A2_1s), GAGAAGCAGCAGCAAGATAGG (BTN3A2_1as), GCAACAGAGCGGGAAATAAG (BTN3A2_2s) and ACGAAGACTCCTCTCCACGA (BTN3A2_2as). Expression of two housekeeping genes (GUSB and ACTB) was used for normalization.