Supplemental Note. The Fragmentation Patterns of Methyl Sterols Identified In the Patient's Plasma and Skin By GC-MS

The numbered peaks correspond to the peaks in Figure 2

Peak 6
unidentified mono methyl sterol
55 (100%), 472 (52%), 227 (25%), 367 (18%), 269 (15%), 457 (14%), 283 (11%), 382 (5%)

Peak 8
methyl cholest 8(9)-en
75 (100%), 472 (87%), 227 (50%), 367 (37%), 269 (29%), 243 (21%), 457 (16%), 282 (10%)

Peak 10*
methyl cholest 7(8)-en
472 (100%), 269 (76%), 382 (40%), 227 (33%), 367 (27%), 243 (23%), 457 (17%)

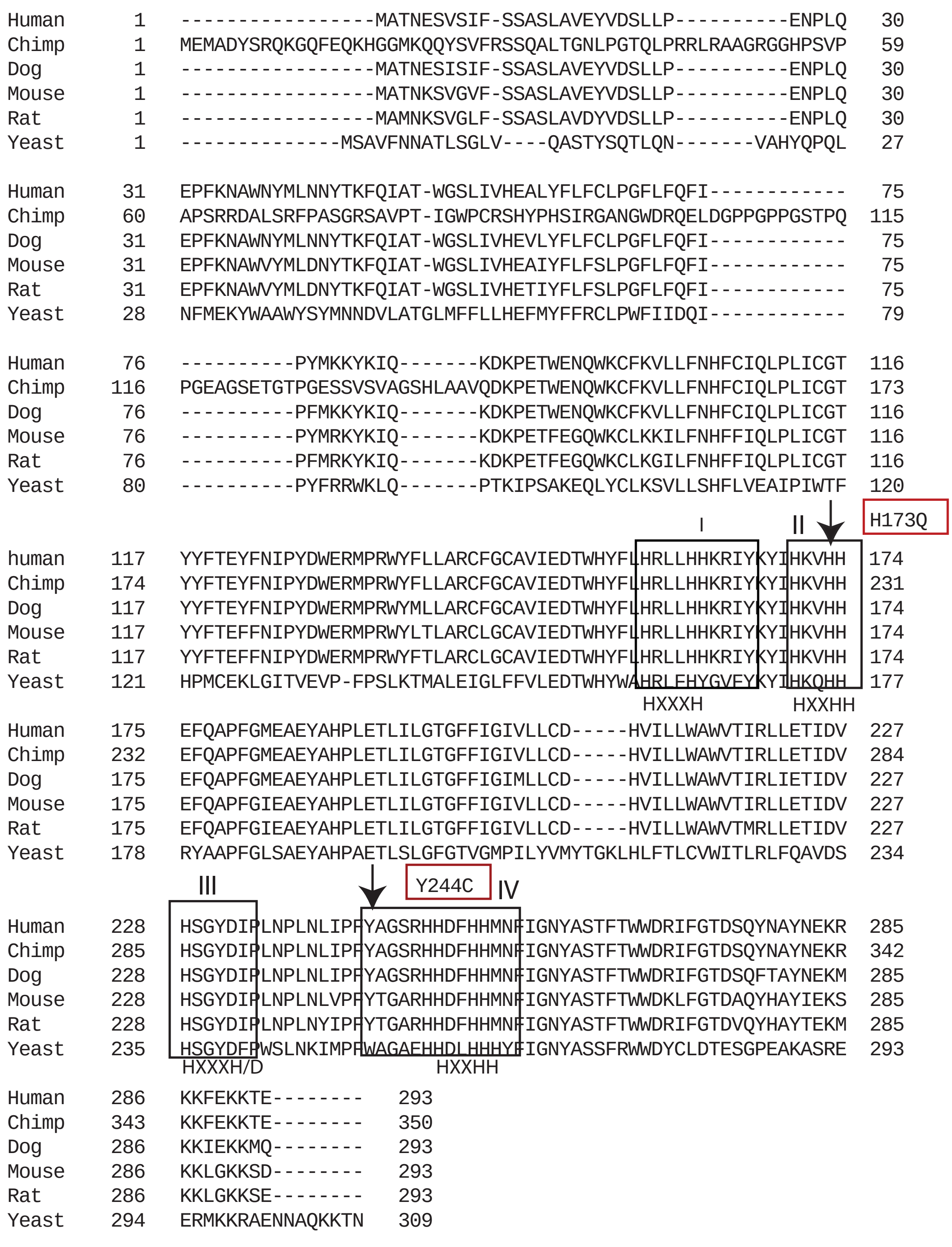
Peak 11
unidentified isomer of peak 12
135 (100%), 486 (22%), 353 (21%), 283 (15%), 381 (12%), 396 (10%), 368 (7%), 458 (6%), 472 (6%)

Peak 12*
4,4-dimethyl cholest 8(9)-en
135 (100%), 486 (25%), 381 (20%), 260 (20%), 283 (11%), 396 (9%), 353 (8%), 471 (4%)
(Hashimoto F, Hayashi H. Biochimica et Biophysica Acta 1086 (1991) 115-124)

Peak 13 **
4,4-dimethyl cholest 8(9), 24-dien
69 (100%), 135 (77%), 484 (22%), 379 (16%), 394 (11%), 351 (6%), 469 (6%)

*The fragmentation pattern of these two peaks were previously published: Hashimoto F, Hayashi H. Biochimica et Biophysica Acta 1086 (1991) 115-124
** The fragmentation pattern of peak 13 is published in the National Institute of Standards (NIST) library

He, etal, (2010) Supplemental Figure 1



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Human      1   -----------------MATNESVSIF-SSASLAVEYVDSLLP----------ENPLQ   30
Chimp      1   MEMADYSRQKGQFEQKHGGMKQQYSVFRSSQALTGNLPGTQLPRRLRAAGRGGHPSVP   59
Dog        1   -----------------MATNESISIF-SSASLAVEYVDSLLP----------ENPLQ   30
Mouse      1   -----------------MATNKSVGVF-SSASLAVEYVDSLLP----------ENPLQ   30
Rat        1   -----------------MAMNKSVGLF-SSASLAVDYVDSLLP----------ENPLQ   30
Yeast      1   --------------MSAVFNNATLSGLV----QASTYSQTLQN-------VAHYQPQL   27

Human     31   EPFKNAWNYMLNNYTKFQIAT-WGSLIVHEALYFLFCLPGFLFQFI------------   75
Chimp     60   APSRRDALSRFPASGRSAVPT-IGWPCRSHYPHSIRGANGWDRQELDGPPGPPGSTPQ  115
Dog       31   EPFKNAWNYMLNNYTKFQIAT-WGSLIVHEVLYFLFCLPGFLFQFI------------   75
Mouse     31   EPFKNAWVYMLDNYTKFQIAT-WGSLIVHEAIYFLFSLPGFLFQFI------------   75
Rat       31   EPFKNAWVYMLDNYTKFQIAT-WGSLIVHETIYFLFSLPGFLFQFI------------   75
Yeast     28   NFMEKYWAAWYSYMNNDVLATGLMFFLLHEFMYFFRCLPWFIIDQI------------   79

Human     76   ----------PYMKKYKIQ-------KDKPETWENQWKCFKVLLFNHFCIQLPLICGT  116
Chimp    116   PGEAGSETGTPGESSVSVAGSHLAAVQDKPETWENQWKCFKVLLFNHFCIQLPLICGT  173
Dog       76   ----------PFMKKYKIQ-------KDKPETWENQWKCFKVLLFNHFCIQLPLICGT  116
Mouse     76   ----------PYMRKYKIQ-------KDKPETFEGQWKCLKKILFNHFFIQLPLICGT  116
Rat       76   ----------PFMRKYKIQ-------KDKPETFEGQWKCLKGILFNHFFIQLPLICGT  116
Yeast     80   ----------PYFRRWKLQ-------PTKIPSAKEQLYCLKSVLLSHFLVEAIPIWTF  120
                                                                      H173Q
                                                          I       II  ↓
human    117   YYFTEYFNIPYDWERMPRWYFLLARCFGCAVIEDTWHYFLHRLLHHKRIYKYIHKVHH  174
Chimp    174   YYFTEYFNIPYDWERMPRWYFLLARCFGCAVIEDTWHYFLHRLLHHKRIYKYIHKVHH  231
Dog      117   YYFTEYFNIPYDWERMPRWYMLLARCFGCAVIEDTWHYFLHRLLHHKRIYKYIHKVHH  174
Mouse    117   YYFTEFFNIPYDWERMPRWYLTLARCLGCAVIEDTWHYFLHRLLHHKRIYKYIHKVHH  174
Rat      117   YYFTEFFNIPYDWERMPRWYFTLARCLGCAVIEDTWHYFLHRLLHHKRIYKYIHKVHH  174
Yeast    121   HPMCEKLGITVEVP-FPSLKTMALEIGLFFVLEDTWHYWAHRLFHYGVFYKYIHKQHH  177
                                                        HXXXH       HXXHH

Human    175   EFQAPFGMEAEYAHPLETLILGTGFFIGIVLLCD-----HVILLWAWVTIRLLETIDV  227
Chimp    232   EFQAPFGMEAEYAHPLETLILGTGFFIGIVLLCD-----HVILLWAWVTIRLLETIDV  284
Dog      175   EFQAPFGMEAEYAHPLETLILGTGFFIGIMLLCD-----HVILLWAWVTIRLIETIDV  227
Mouse    175   EFQAPFGIEAEYAHPLETLILGTGFFIGIVLLCD-----HVILLWAWVTIRLLETIDV  227
Rat      175   EFQAPFGIEAEYAHPLETLILGTGFFIGIVLLCD-----HVILLWAWVTMRLLETIDV  227
Yeast    178   RYAAPFGLSAEYAHPAETLSLGFGTVGMPILYVMYTGKLHLFTLCVWITLRLFQAVDS  234
                  III            ↓  Y244C  IV
Human    228   HSGYDIPLNPLNLIPFYAGSRHHDFHHMNFIGNYASTFTWWDRIFGTDSQYNAYNEKR  285
Chimp    285   HSGYDIPLNPLNLIPFYAGSRHHDFHHMNFIGNYASTFTWWDRIFGTDSQYNAYNEKR  342
Dog      228   HSGYDIPLNPLNLIPFYAGSRHHDFHHMNFIGNYASTFTWWDRIFGTDSQFTAYNEKM  285
Mouse    228   HSGYDIPLNPLNLVPFYTGARHHDFHHMNFIGNYASTFTWWDKLFGTDAQYHAYIEKS  285
Rat      228   HSGYDIPLNPLNYIPFYTGARHHDFHHMNFIGNYASTFTWWDRIFGTDVQYHAYTEKM  285
Yeast    235   HSGYDFPWSLNKIMPFWAGAFHHDLHHHYFIGNYASSFRWWDYCLDTESGPEAKASRE  293
                HXXXH/D           HXXHH

Human    286   KKFEKKTE--------   293
Chimp    343   KKFEKKTE--------   350
Dog      286   KKIEKKMQ--------   293
Mouse    286   KKLGKKSD--------   293
Rat      286   KKLGKKSE--------   293
Yeast    294   ERMKKRAENNAQKKTN   309
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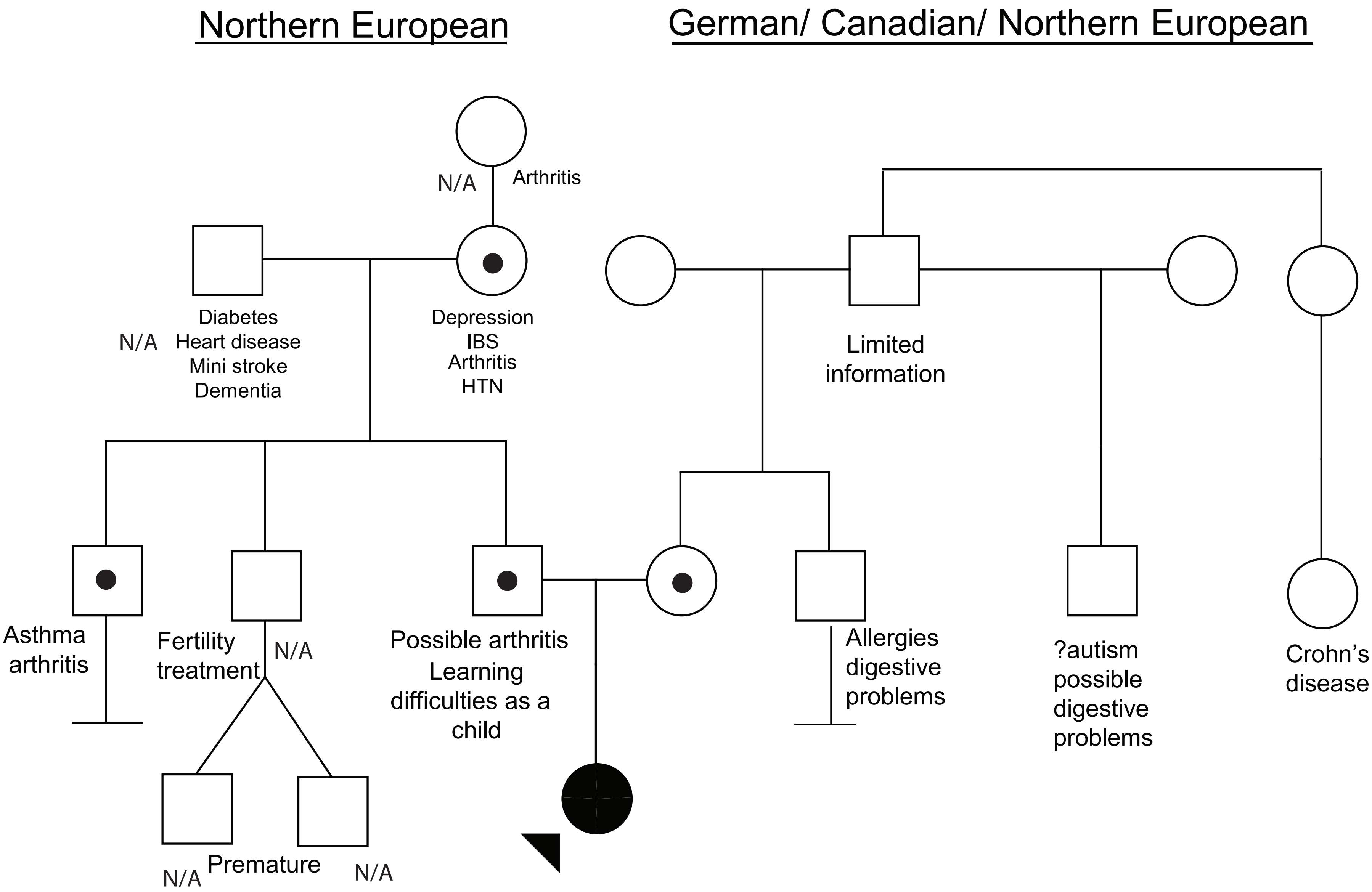
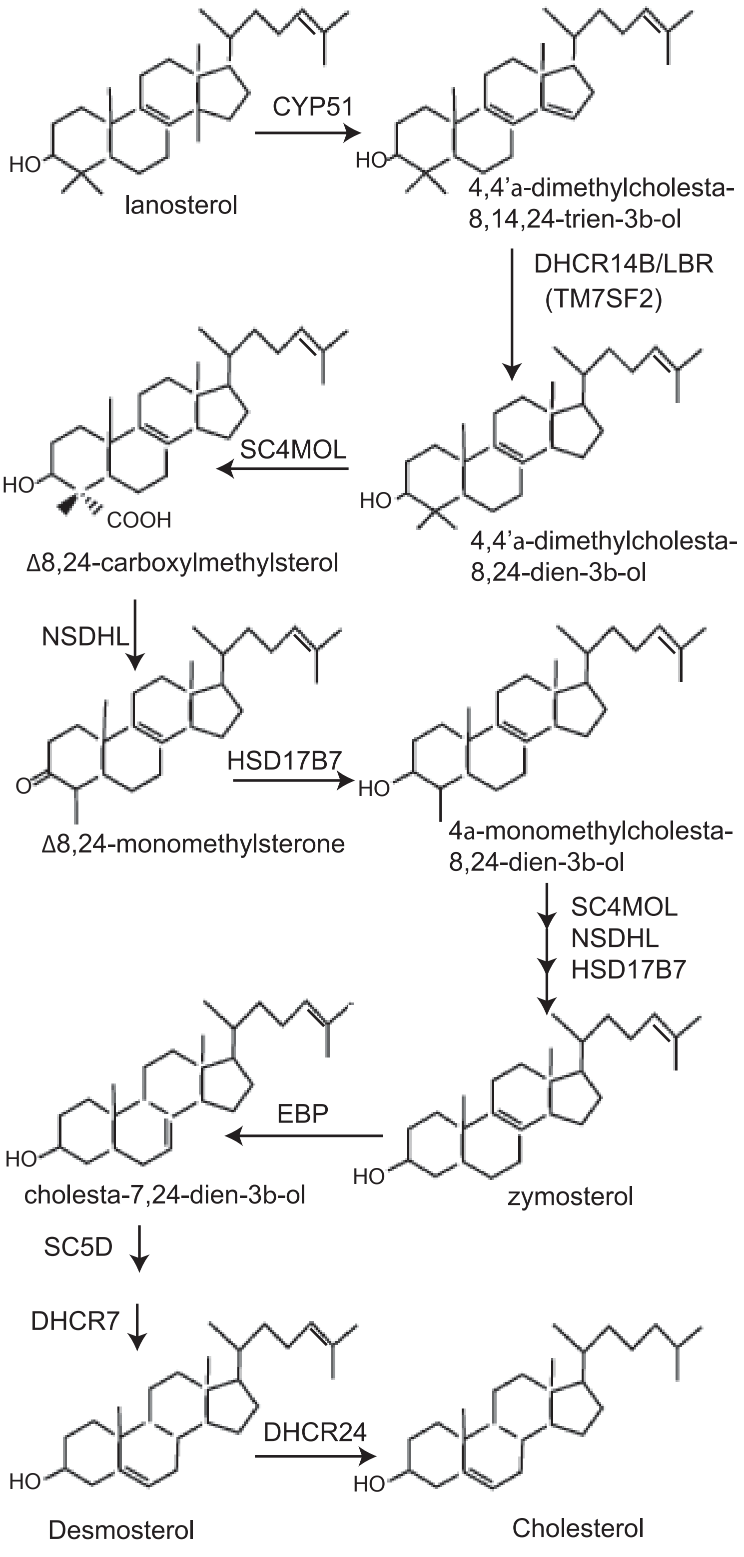
Supplemental Figure 1. Alignment of sterol methyl oxidase protein sequences across evolution. From yeast to human, SC4MOL is highly conserved. Four conserved metal-binding domains were identified and are highlighted with black squares. The conserved metal binding motifs HXXXH/D and HXXHH are marked under the alignment. 519T>A changes amino acid H173 to Q, which replaces the histidine at an active site of the second metal binding domain with glutamine; 731A>G changes amino acid Y244 to C; Y244 is in the fourth metal binding domain and also is conserved across evolution.



Supplemental Figure 2. Paternal family history is significant for early onset of arthritis. Patient's father, one paternal uncle, paternal grandmother, and paternal, maternal great grandmother all have arthritis that started in their late 30s or early 40s. Only one paternal uncle and the paternal grandmother were available for analysis, and both of them were found to have the 519T>A mutation. Maternal family members were unavailable for studies. N/A: sample not available.

He, et al, (2010), Supplemental Figure 3



Supplemental Figure 3. Diagram of the enzymatic pathway for conversion of lanosterol to cholesterol. The saturation of the C-24 double bond can occur at multiple points in the pathway. Both DHCR14B/LBR and TM7SF2 encode proteins with sterol 14 reductase activity.

Supplementary Table 1. Surface markers used in flow cytometry assays

Fluorescence / Sample	Pacific Blue	Pacific Orange	FITC	PE	PerCp-Cy5.5	PE-Cy7	APC	APC-Cy7	Purpose
1	CD3	CD8	TLR-2	TLR-9	CD4	CD56	TLR-4	CD16	Comparison of T-cell lymphocytes and NK cell subpopulations expressing NK cell marker CD56 and/ or TLR's
2	CD3	-	CD86	CD27	-	HLA-DR	CD40	CD20	B-Cell activation and expression of HLA-DR
3	CD3	-	IgG	CD27	-	-	IgM	CD20	B-Cell expression of immunoglobulin B-cell receptor isotypes (B-cell maturation)
4	CD3	CD8	CD16	CD16b	CD4	CD56	CD69	CD25	Comparison of activation among all cell types
5	CD127	-	CD80	CD62L	-	CD3	CD69	CD25	Measurement of granular cell activation, subtype discrimination
6	CD127	-	CD14	TLR-9	-	CD3	CD69	CD25	Measurement of granular cell activation, subtype discrimination, and TLR expression
7	CD127	-	CD66	CD14	-	CD3	CD69	CD25	Granular cell activation, subtype discrimination
8	CD127	-	TLR-2	CD14	CD3	CD69	TLR-4	CD25	Granular cell activation, subtype discrimination, and TLR expression

Supplementary Table 2. Comparative analysis of granulocyte and T cell phenotypes between patient, her father, and healthy controls by multicolor flow cytometry.

Cell Population	**Patient**	**Father**	**Healthy Control**[1]
Granulocytes[2]			
% CD16^{bright} (GMFI[3])	93.3 (16317)	84.6 (24889)	97.5 (34540)
% CD16^{dim} (GMFI)	6.35 (1044)	12.3 (1706)	1.68 (1403)
% CD16b (GMFI)	44.1 (185)	86.4 (338)	99.5 (1074)
% CD25+ (GMFI)	43.3 (3921)	27.8 (3126)	10.8 (473)
% CD69+ (GMFI)	34.6 (1265)	23.7 (1094)	6.81 (120)
% CD86+ (GMFI)	33.4 (9421)	22.4 (8488)	20.7 (25)
% HLA-DR+ (GMFI)	31.3 (1235)	21.9 (998)	10.8 (708)
% TLR-2+ (GMFI)	30.7 (2756)	16.8 (2437)	5.63 (1785)
% TLR-4+ (GMFI)	0.80 (724)	1.40 (769)	87.2 (1010)
T cells[4]			
CD4 subset (% of total CD3+)	50.7	40.3	64.3
% CD28+ (GMFI)	99.9 (1031)	99.9 (1127)	98.0 (1689)
% CD56+ (GMFI)	0.67 (1214)	0.66 (1233)	0.29 (1101)
CD8 subset (% of total CD3+)	41.9	40.4	26.2
% CD28+ (GMFI)	87.4 (605)	86.8 (694)	71.4 (2171)
% CD56+ (GMFI)	10.8 (3318)	14.3 (2298)	6.32 (2889)
CD8^{dim} (% of total CD3+)	4.12	6.58	1.9
% CD28+ (GMFI)	55.8 (817)	56.9 (774)	54.5 (1280)
% CD56+ (GMFI)	43.5 (3152)	48.5 (2285)	25.7 (2870)

[1] Values shown are means of 20 controls; with standard deviations within 15% of the mean.

[2] Granulocyte gate A in Figure 4d; CD3$^-$CD20$^-$CD16$^+$ cells.

[3] GMFI, geometric mean fluorescence intensity of the indicated antigenic marker. All GMFI values shown have robust coefficients of variation less than 1%.

[4] Lymphocyte gate C in Figure 4d; CD3$^+$CD20$^-$CD16$^-$ cells.

Supplementary Table 3. Selected Serum cytokine levels (pg/ml) in patient and her father using multiplex bead array*

Proinflammatory	**IL-1β**	**IL-6**	**IL-8**	**TNF-α**	**GM-CSF**	**G-CSF**
Patient	1.5	9.1	9.1	0	7.8	26.3
Father	0.2	0	1.8	0	1.3	25.1
Th1/Th2 distinguishing	**IFN-γ**	**IL-2**	**IL-4**	**IL-5**	**IL-10**	**IL-12**
Patient	91.2	35.5	2.2	0	1.1	0
Father	44	0	0.6	0	1.5	0.48
Chemokines	**MCP-1**			**MIP-1β**		
Patient	23.8			10		
Father	17.1			41.2		

* Serum cytokine levels in healthy controls are generally below the detection level

Supplemental Table 4. Cellular Cholesterol Levels In Patient and Control Fibroblasts Under Variable Growth Conditions

Fibroblasts*	**Culture Conditions**			
	Cholesterol Restriction	**Statin**	**10% FBS**	**Cellular Cholesterol** ***(mg/mg protein)***
SC4MOL def	3 Days	–		0.31, 0.62
	7 Days	–		1.15, 0.98
	7 Days	+		0.88, 0.64
	None		+	1.49, 2.25
Control	3 Days	–		0.88, 1.08
	7 Days	–		2.13, 2.05
	7 Days	+		0.76, 1.08
	None		+	0.75, 0.96

* Data from duplicated experiments are shown.