

Mutations in the *SC4MOL* gene encoding a novel methyl sterol oxidase cause autosomal recessive psoriasisiform dermatitis, microcephaly and developmental delay

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Summary

Disorders of cholesterol biosynthesis have clinical manifestations involving skeleton, eyes, neurologic development, and skin¹. We describe a patient with congenital cataracts, developmental delay, microcephaly, and low serum cholesterol who developed severe psoriasisiform dermatitis and arthralgias beginning at age 3. Her brain MRI indicated minor gliosis. Quantitative sterol analysis of patient plasma and skin showed marked elevation of 4 α -methyl- and 4, 4'-dimethylsterols, indicating a deficiency in the first step of sterol C4 demethylation in cholesterol biosynthesis. Molecular studies showed mutations in *SC4MOL*, a gene predicted to encode a sterol C4 methyl oxidase. Thus, our patient has a previously undescribed inborn error of cholesterol biosynthesis. Cellular studies with patient-derived fibroblasts showed higher mitotic rate than control cells in cholesterol-depleted medium, in which *de novo* cholesterol biosynthesis was increased with the accumulation of methylsterol. Immunologic analyses showed dysregulation of immune-related receptors in the patient and her father. Inhibition of sterol C4 methyl oxidase in human transformed lymphoblasts or in fresh leukocytes induced activation of cell cycle, and immune receptor dysregulation. These findings suggest that methylsterols influence mitotic capacity and immune function. *SC4MOL* is situated within the psoriasis susceptibility locus *PSORS9*², and is likely a genetic risk factor for common psoriasis.

The patient was seen in our clinic at the age of 13. She had a severe dermatitis affecting her entire body, sparing the palms (**Fig 1a-c**). Skin biopsy was characteristic of psoriasisiform dermatitis (**Fig 1d**). While her skin briefly improved with cyclosporine A treatment, she did not respond to other treatments including topical corticosteroids, dovonex, etanercept, phototherapy, and oral isotretinoin. The refractory nature of her skin disease, and mild developmental and growth delay with delayed skeletal maturation, a history of multiple allergies, mild arthritis, and frequent infections, prompted further evaluation. She had markedly elevated levels of serum IgE at 1,486 IU/ml (normal <180) and IgA at 684 IU/ml (normal <218). Her serum lipid profile showed only a persistently low total cholesterol level (**Fig 2c**).

A cholesterol biosynthesis defect was suspected. Analysis of plasma sterols by gas chromatography-mass spectrometry (GC-MS) showed 20- and 500-fold elevation of 4 α -monomethyl sterols and 4, 4'-dimethyl sterols, respectively (**Fig 2a**). This pattern of elevation has not been previously associated with a defect in the sterol synthesis pathway, but was consistent with a defect in a methylsterol oxidase.

Therefore, we sequenced the *SC4MOL* gene that was predicted to encode this enzyme. Two variations from the published sequence were identified; 519T>A and 731A>G encoding H173Q and Y244C, respectively, in the sterol C4 methyl oxidase-like protein (**Fig 3a**), herein referred to as sterol methyl oxidase (SMO). The 519T>A mutation was carried by the patient's father, and the 731A>G was carried by the mother. Both amino acids occur within highly conserved metal-binding domains in SMO (**Fig 3b**). In addition, H173Q alters a predicted active site of the second iron-binding motif of the enzyme.

Plasma methylsterol levels were also elevated in the patient's parents (**Fig 2c**). This suggests a subclinical effect for heterozygotes, in keeping with that seen in carriers of a *DHCR14B/LBR* mutation who have altered nuclear morphology in granulocytes (Pelger-Huët anomaly)³. *DHCR14B/LBR* encodes C14-sterol reductase, immediately upstream of SMO, which also functions as a nuclear lamin B receptor⁴. Tight regulation of these two enzymes in the pathway underscores extra-sterol functions of 4, 4'-dimethylsterols.

The two most abundant dimethylsterols; 4, 4'-dimethyl-5 α -cholesta-8,24-dien-3 β -ol (testis-meiosis activating sterol (MAS)), and 4, 4'-dimethyl-5 α -cholesta-8, 14, 24-trien-3 β -ol (follicular fluid -MAS), substrates for SMO and C14-sterol reductase respectively, are members of the MAS family and are found in high concentration in testis and ovary. The underlying mechanism of their extra-sterol function remains unknown. However since 4, 4'-dimethylsterols are either direct substrates or products of C14 sterol reductase, they could function as signaling sterols for nuclear lamin B receptor. The nuclear lamin B receptor is important for nuclear membrane breakdown during meiosis of germinal cells^{5,6} and formation of the mature granulocyte nucleus⁷. Granulocyte/neutrophil-rich pathology is characteristic of psoriasis and psoriatic arthritis^{8,9}. Thus the mild elevation of 4, 4'-dimethylsterols seen in the patient's father could be relevant to his history of mild inflammatory joint disease and a paternal family history of early onset inflammatory joint disease (**Supplementary Fig 1** online).

Skin cell hyperproliferation causes flaking skin, characteristic of psoriasis. All known genetic disorders that cause increased levels of MASs, including defects in *DHCR14/LBR*, *NSDHL* and *EBP* (**Fig 2b**)^{1,10,11}, present with ichthyosiform/psoriasiform dermatitis in humans and mice. However, none of the defects adjacent or distal to MAS demethylation complex, including *CYP51*, *SC5D*, or *DHCR7*, manifest skin lesions¹²⁻¹⁴. Previous studies of germinal cells have demonstrated meiosis-activating function of MAS^{15,16}, but the effect of MAS on cell cycle regulation and mitosis is unknown.

Therefore, we examined cell proliferation in patient skin fibroblasts (**Fig 4a-c**). The rate of cell division was found to be higher in patient fibroblast than control cells cultured in cholesterol-depleted medium, a condition under which *de novo* cholesterol biosynthesis is stimulated (**Fig 4a**). The S-G2-M to G0-G1 ratio in patient skin fibroblasts was >3-fold higher than control cells (**Fig 4b**). This ratio peaked after 2-3 days growth in the cholesterol-depleted medium, and corresponded with peaks of cellular methylsterols and total protein (**Supplementary Table 1** online).

Growth of patient cells in cholesterol-depleted suggest association between MAS and cell division. To further examine this possibility, transformed human lymphoblasts were cultured in the presence of a SMO inhibitor, 3-amino-1,2,4-triazole (ATZ). Under these conditions, the cells accumulated a profile of methylsterols similar to that of the patient's lymphocytes grown without ATZ (data not shown). Parallel to this, the S-G2-M/G0-G1 ratio increased 3-fold in treated lymphoblasts, while neither simvastatin or fluconazole (an inhibitor of lanosterol 14 α demethylase (*CYP51*)) had a significant effect on cell cycle progression (**Fig 4c**). These data indicate alterations in SMO that in turn affects cell cycle activation.

Patients with genetic defects in *NSDHL*, which encodes the enzyme immediately downstream of SMO, occasionally present with symptoms nearly identical to those with defects in *EBP*^{1,10,11} and *vice versa*; but they always differ in skin histopathology. Out of newborn period, *NSDHL*-deficient patients have psoriasiform dermatitis whereas *EBP*-deficient patients have ichthyosis, a condition associated with minor skin inflammation. Our patient, and *NSDHL*-deficient patients accumulate 4, 4'-dimethylsterols in their skin, but *EBP*-deficient patients do not. This suggests that dimethylsterols differ from other MAS in contributing to the perturbation of immune function. *SC4MOL* is located on 4q32-34, the known psoriasis susceptibility locus *PSORS9* (4q31-34). Histologically, our patient's dermatitis is identical with that seen in classical psoriasis.

Since both the patient and her father had a significant elevation of dimethylsterols, we examined a comprehensive panel of immunologic parameters as described in the methods and **Supplementary Table 2** online. Selected multicolor flow cytometry profiles of granulocytes and T cells are shown in **Figure 4d**, and the geometric mean fluorescence intensity, and percentage of cells for each measurement are shown in **Supplementary Table 3** online.

We found that activated CD16⁺ granulocytes; identified by CD25⁺CD69⁺ and CD86⁺HLA-DR⁺ subsets; were increased 30- and 20-fold in the patient and father, respectively, compared with healthy controls (**Fig 4d** and **Supplementary Table 3** online). In addition, there was 30- and 15-fold increased numbers of toll-like receptor (TLR)-2⁺TLR-4⁻ granulocytes in the patient and father, respectively, compared with healthy controls (**Fig 4d**). Up-regulation of TLR-2, but not TLR-4, is considered typical for patients with psoriasis¹⁷ or psoriatic arthritis¹⁸. Over-expression of TLR-2 and down-regulation of TLR-4 on granulocytes suggest perturbation of innate immune responses to bacterial infection. Consistent with this observation, expression of granulocyte-specific, CD16b isoform was also markedly down-regulated in both patient and father (**Fig 4e**), suggesting a defect in phagocytic function.

In the lymphocyte compartment, both patient and father had significantly higher proportions of CD8^{dim} T cells that were also CD28^{null}CD56⁺ (**Fig 4d**, **Supplementary Table 3**). Down-regulation of CD8 and CD28, and accumulations of CD28^{null}CD56⁺ T cells are indicative of pervasive immune activation in the setting of chronic inflammatory disease¹⁹.

We then examined whether inhibiting SMO by ATZ affected normal leukocytes. Results showed over 6-fold increase in TLR-2⁺TLR-4⁻ granulocyte population (**Fig 4f**), and 2-fold decrease in CD8 expression in normal lymphocytes (**Supplementary Table 4** online). These results recapitulated the *in vivo* granulocyte and T cell phenotypes of the patient and father (**Fig 4d**). All these data indicate a link between cholesterol homeostasis and immunity.

Because psoriasis and psoriatic arthritis are known to be driven by the production of certain cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-6, the serum cytokine profiles of the patient and father were investigated by multiplex assay (**Supplementary Table 5** online). Consistent with the flow cytometry data, a number of cytokines and chemokines associated with granulocyte and monocyte activation were elevated in the patient, and to a lesser extent in her father. This included granulocyte and monocyte colony stimulation factor, which is known to trigger psoriasiform eruption in humans²⁰. The patient also had elevated IL-6 and IL-8 (**Supplementary Table 5** online), cytokines that amplify skin inflammation as has been demonstrated in psoriatic skin and fibroblasts²¹.

The classical pro-inflammatory cytokine TNF- α was normal in the patient, consistent with the fact that her dermatitis did not respond to treatment with etanercept. Cultures of patient skin fibroblasts showed constitutive production of IL-6 compared to control fibroblasts regardless of passage number (**Fig 4g**), and were further increased with the addition of recombinant TNF- α , a known inducer of IL-6. To investigate a possible association of the defect in cholesterologenesis and cytokine production, we treated control and patient fibroblasts with simvastatin, which blocks cholesterol biosynthesis upstream of SMO. After 24 hours, IL-6 production by patient fibroblasts was significantly decreased even in the presence of exogenous TNF- α (**Fig 4g**), suggesting that the accumulated sterol intermediates in our patient were stimulating IL-6 production.

In summary, we have identified the first patient deficient in SMO, a novel enzyme in the C4 methylsterol demethylation complex that is encoded by *SC4MOL*. Our data suggest a role for SMO in regulating granulocyte activation, CD8 expression on T cells, cytokine production, and cell proliferation, factors that are critical in the pathogenesis of common psoriasis. We have previously reported elevated methylsterols in skin flakes from patients with common psoriasis²². Thus variation in expression or function of SMO may be an important modifying factor in the development of common psoriasis.

Methods

This study was conducted pursuant to an IRB protocols approved by the University of Pittsburgh.

Genomic DNA and cDNA amplification and sequencing. Total RNA and gDNA was extracted from patient fibroblasts for reverse transcription, PCR amplification, and sequencing as described²³. Six exons of *SC4MOL* were amplified using intronic primers (all primer sequences available upon request).

Sterol analyses. Sterol distribution was measured in cultured skin fibroblasts, transformed lymphocytes, serum, or skin flakes obtained from patients and controls by GC and selected-ion MS as described^{11,24}.

Flow cytometry on peripheral blood cells

Flow cytometry on cells from patient and controls were carried out and analyzed as described previously^{19,21}. In the sterol oxidase inhibition studies, 10 ml of erythrocyte-depleted blood from a healthy 16-year old girl was incubated with 5mM ATZ for 60 hours at 37 degree with 5% CO₂, both ATZ treated and untreated total leukocytes were then isolated and stained as described in Supplementary Table 2 online.

Serum cytokine quantification. Cytokine levels were determined by multiplex assay using the Luminex system (Luminex Corporation) as described previously^{25,26}.

Cell culture, proliferation and ELISA.

Primary dermal fibroblasts were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin and GlutaMAX (Invitrogen), TNF- α (10ng/ml) and simvastatin (5 μ M) were added to fibroblasts at 80% confluency, and culture media were collected after 24 hours for measurement of IL-6 using an ELISA kit (BD Bioscience). For cell proliferation assays, primary fibroblasts or EBV-transformed human lymphoblasts were cultured with cholesterol-depleted medium as described²⁴; and ATZ (2mM), simvastatin (5 μ M) or fluconazole (12.5 μ M) were added to the medium before the cells were subjected to the proliferation assay.

Cell proliferation and cell cycle stage analysis

Skin fibroblasts, or EBV-transformed lymphoblast (2 x 10⁷ cells/ml) were labeled with 0.25 μ M of 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes) using established procedures²⁰. At different days of culture, cells were harvested and stained with 4',6'-diamidino-2-phenylindole (DAPI), and analyzed by multicolor flow cytometry. The number of cell divisions was determined by the number of CFSE fluorescence peaks. The number of cells in G0-G1 and in the S-G2-M phases of the cell cycle was proportional to the amount of linear DAPI fluorescence, G0-G1 cells having half the amount of DAPI staining as the S-G2-M cells.

Figure Legend

Figure 1. *Severe, scaling, and psoriasiform dermatitis in the patient.* **a.** Note mild microcephaly, lusterless fine fair hair, and blepharitis on face. **b** and **c.** The ichthyosiform erythroderma covers all of the patient's body except for the palms and soles. **d.** H&E-stained section of affected skin shows hyperkeratosis, loss of granular layer, psoriasiform hyperplasia, thinning of suprapapillary plate, and neutrophilic epidermal infiltration; these features are characteristic of psoriasis. Photograph is shown per signed informed consent/assent provided by the patient and her family.

Figure 2. *Sterol profiles of patient samples and diagram of the sterol C4 demethylation pathway.* **a&b.** Gas chromatographic flame ionization profiles of sterol extracts of patient skin (a) and plasma (b). The ordinates are detector response and elution time. The identified compounds are: 1, internal standard (epicoprostanol); 2, cholesterol; 3, lathosterol; 4, phytosterol; 5, campesterol; 6, 4 α -methyl-5 α -cholest-8(9)-en-3 β -ol; 7, 4 α -methyl-5 α -cholest-7(8)-en-3 β -ol; 8, 4, 4'-dimethyl-5 α -cholesta-8(9)-en-3 β -ol; 9, lanosterol; 10, sitosterol; 11, 4, 4'-dimethyl-5 α -cholesta-8(9),24-dien-3 β -ol. Both dimethylsterols and monomethylsterols were markedly elevated in patient skin and plasma. Dimethylsterol (peak 8) is most elevated in skin suggesting the preferential accumulation of 4, 4'-dimethylsterols in the patient's skin compared to plasma, which may explain the severe involvement of the patient's skin. The absence of a 4-carboxysterol in the skin excludes a possible defect in *NSDHL*. **b.** Diagram of the enzymatic pathway for conversion of lanosterol to cholesterol. The saturation of the C-24 double bond may occur at different points in the pathway. The physiological function *TM7SF2* is unclear²⁷. **c.** Plasma sterol analysis and plasma total cholesterol levels from controls, the patient before cholesterol supplementation and after 3 months of cholesterol supplementation, and the patient's mother and father. Methylsterols levels are the sum of both dimethylsterols and monomethylsterols. After 3 month of cholesterol supplementation, a 19% reduction in the patient's plasma methylsterol level was seen, together with an increase of her total cholesterol level. After 6 month of cholesterol supplementation, the patient's total cholesterol normalized, however no further reduction of methylsterols was seen (data not shown).

Figure 3. *Mutation analysis of SC4MOL in proband and parental samples, and the alignment of amino acid sequences of SMO among different species.* **a.** Two mutations were identified in *SC4MOL* from both gDNA and cDNA extracted from peripheral blood leukocytes, T519>A and A731>G. T519>A was also identified in gDNA from patient's father, and A731>G was identified in her mother. **b.** Alignment of SMO 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 HXXXHH are marked under the alignment. T519>A changes amino acid H173 to Q, which replaces the histidine at an active site of the second metal binding domain with glutamine; A731>G changes amino acid Y244 to C; Y244 is in the fourth metal binding domain and also is conserved across evolution.

Figure 4. *Fibroblast and immunocyte abnormalities in the patient and her father.*

a,b: Fibroblasts were labeled with CFSE and DAPI, cultured, and analyzed by flow cytometry. The number of cell divisions was determined by the number of CFSE fluorescence peaks. The number of cells in G0-G1 and in the S-G2-M phases of the cell cycle was analyzed by the amount of linear DAPI fluorescence. **c** Histograms show typical DAPI fluorescence profiles of cells in G0-G1 and G2-S-M phases of the cell cycle in human EBV-transformed lymphoblasts cultured in standard FBS-supplemented media (FBS); or in cholesterol-depleted medium (CD); or in CD supplemented with either simvastatin (ST), 3-amino-1,2,4 triazole (ATZ), or fluconazole (FA). **d,e:** Leukocyte populations were analyzed by multicolor flow cytometry; subsets identified by forward/scatter profiles, with mature granulocytes, monocytes, and lymphocytes falling in gates A, B, and C, respectively. Data shown are cytometric profiles for CD25, CD69, CD86, HLA-DR, TLR-2, and TLR-4 (**d**), and fluorescence histograms of CD16b (**e**) for mature granulocytes/neutrophils. Also shown are the CD4, CD8, profiles of CD3⁺ T cells; and CD28^{null} CD56⁺ in CD8^{dim} T cells (gate D). No significant differences were observed in the monocyte population (data not shown). **f.** Cytometric profiles for TLR-2 and TLR-4 on granulocytes of healthy control with (blue) or without (red) ATZ treatment. Overlay of cytograms show ATZ-induced phenotypic shift towards increased TLR-2 expression. **g;** IL-6 production by dermal fibroblasts from healthy control and patient upon treatment of TNF- α , and TNF- α plus simvastatin, or in media alone.

Acknowledgements

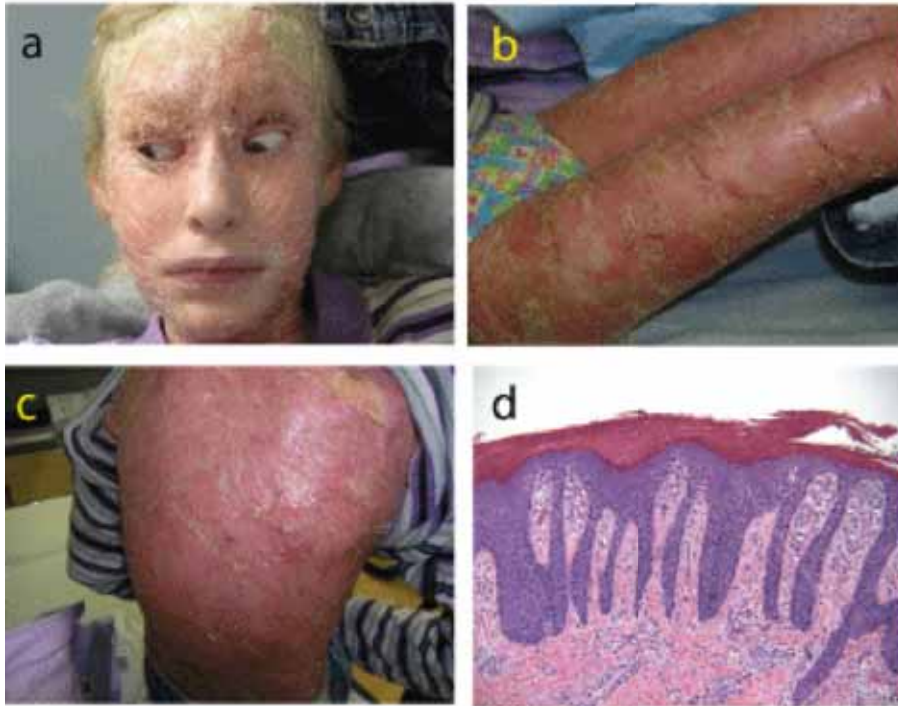
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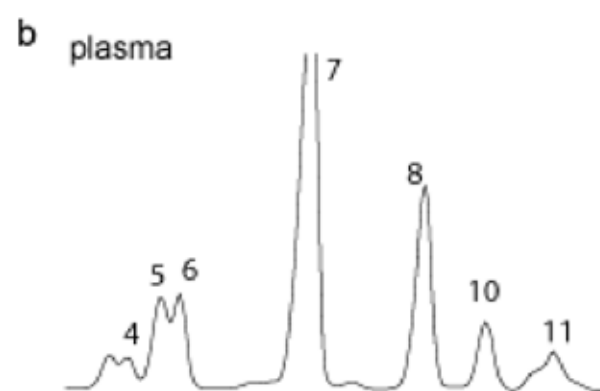
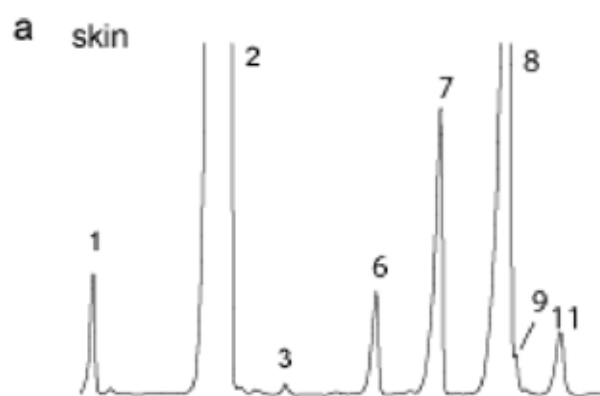
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Figure 1, He, et al., 2008





c

	Methyl sterols ($\mu\text{g/ml}$)	Total Cholesterol (mg/dl)
Normal	3.0 ± 2	140-176
Patient	43	85
Patient + Cholesterol	31	124
Mother	6.4	188
Father	18.5	231

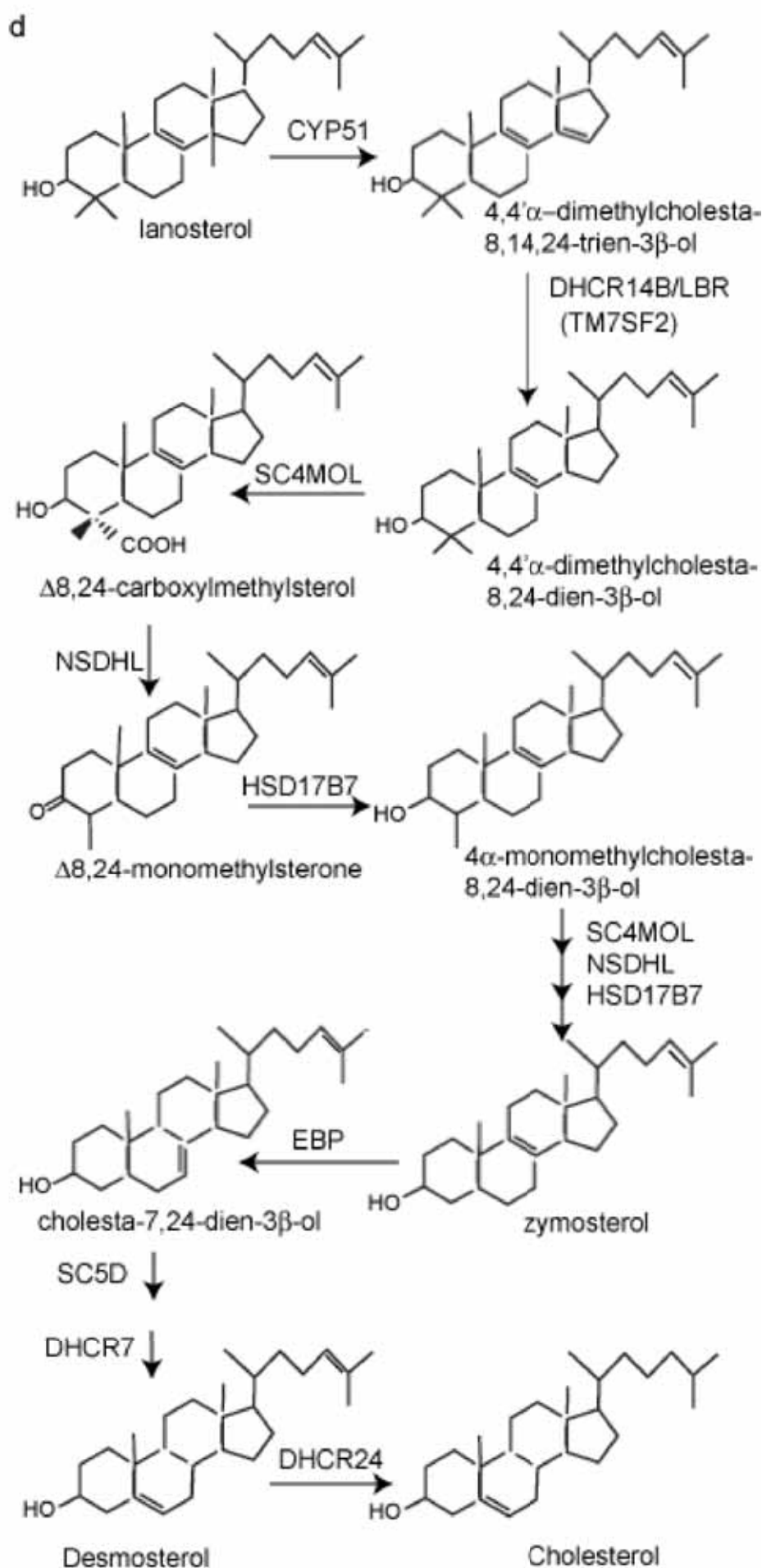


Figure 3, He, et al., 2008

b

Human	1	-----MATNESVISIP-SSASLAVEYVDSLPLP-----ENPLQ	30
Chimp	1	MEMADYSRQKGQFEQKHGKMKQYVFRSSQALTCNLPGTQLPRRLRAGRGHPSPV	59
Dog	1	-----MATNESISIF-SSASLAVEYVDSLPLP-----ENPLQ	30
Mouse	1	-----MATNKSQGVF-SSASLAVEYVDSLPLP-----ENPLQ	30
Rat	1	-----MAMKSVGLF-SSASLAVDYVDSLPLP-----ENPLQ	30
Yeast	1	-----MSAVFNATLSGLV----QASTYSQTLQN-----VAHYQPQL	27
Human	31	EPFKNAWYMLNNTKQIAT-WGSLIVHEALYFLCLPGFLQFI-----	75
Chimp	60	APSRDALSRFPASGRSAVPT-IGWPCSHYPHSIRGANGNDRQELDGGPPGPTPQ	115
Dog	31	EPFKNAWYMLNNTKQIAT-WGSLIVHEVLYFLCLPGFLQFI-----	75
Mouse	31	EPFKNAWYMLDNYYTKQIAT-WGSLIVHEALYFLSPLPGFLQFI-----	75
Rat	31	EPFKNAWYMLDNYYTKQIAT-WGSLIVHETIYFLSPLPGFLQFI-----	75
Yeast	28	NFMEKYMAAWYSYMNNDVLAGLMEFFLLHSEFNFFRCPLPWFIIIDQI-----	79
Human	76	-----PYMKYKIQ-----KDKPETWENQWCKFKVLLFNHFICQLPLICGT	116
Chimp	116	PGEAGSETGPGESSVAVGSHLAAVQDKPETWENQWCKFKVLLFNHFICQLPLICGT	173
Dog	76	-----PFMKYKIQ-----KDKPETWENQWCKFKVLLFNHFICQLPLICGT	116
Mouse	76	-----PYMKYKIQ-----KDKPETFGQWCKLKKILFNHFFIQLPLICGT	116
Rat	76	-----PFMRKYKIQ-----KDKPETFGQWCKLKKILFNHFFIQLPLICGT	116
Yeast	80	-----PYPRRWKLQ-----PTKIPSAKQYCLKSVLLSHLFLVEAIPWTF	120
human	117	YYFTEYENIPYDWERMPRWYFLLARCFGCVAIEDTNEFLHRLLLHHRKIKYIHKVHR	174
Chimp	174	YYFTEYENIPYDWERMPRWYFLLARCFGCVAIEDTNEFLHRLLLHHRKIKYIHKVHR	231
Dog	117	YYFTEYENIPYDWERMPRWYFLLARCFGCVAIEDTNEFLHRLLLHHRKIKYIHKVHR	174
Mouse	117	YYFTEFNIIPYDWERMPRWYFLLARCLGCAVIEDTNEFLHRLLLHHRKIKYIHKVHR	174
Rat	117	YYFTEFNIIPYDWERMPRWYFLLARCLGCAVIEDTNEFLHRLLLHHRKIKYIHKVHR	174
Yeast	121	HFMCEKIGITVEVP-FPSLAKTNALSIGLFFVLEDTNE ^{HXXXH} MAHRLFHYGVFNKYIHKQHR	177
Human	175	EFOAPFGMBABAYAHPLETLILGTGFFIGIVLLCD-----HVILLMAWVTIRLLETIDV	227
Chimp	232	EFOAPFGMBABAYAHPLETLILGTGFFIGIVLLCD-----HVILLMAWVTIRLLETIDV	284
Dog	175	EFOAPFGMBABAYAHPLETLILGTGFFIGIMLLCD-----HVILLMAWVTIRLLETIDV	227
Mouse	175	EFOAPFGIBABAYAHPLETLILGTGFFIGIVLLCD-----HVILLMAWVTIRLLETIDV	227
Rat	175	EFOAPFGIBABAYAHPLETLILGTGFFIGIVLLCD-----HVILLMAWVTIRLLETIDV	227
Yeast	178	RYAAPFGLSABAYAHPAETLSLPGTVGMPIYVMYTGKHLHLFTLCVWITLRLPQAVDS	234
Human	228	HSGYDIPLMPLNLIIPRYAGSRHHDFHRMNFIGNYASTFTWDRIFGTDSCQYAYNEKR	285
Chimp	285	HSGYDIPLMPLNLIIPRYAGSRHHDFHRMNFIGNYASTFTWDRIFGTDSCQYAYNEKR	342
Dog	228	HSGYDIPLMPLNLIIPRYAGSRHHDFHRMNFIGNYASTFTWDRIFGTDSPQYAYNEKM	285
Mouse	228	HSGYDIPLMPLNLVPRYTGARHHDFHRMNFIGNYASTFTWDXKLFGTDQYHAYIEKS	285
Rat	228	HSGYDIPLMPLNYIPRYTGARHHDFHRMNFIGNYASTFTWDRIFGTDVQYHAYTERM	285
Yeast	235	HSGYDFPWSLNKIMPRMAGARHHDFHRMNFIGNYASSFRWDYCLDTSRGPSEAKASRE	293
Human	286	KKFEKTE-----	293
Chimp	343	KKFEKTE-----	350
Dog	286	KKIEKMQ-----	293
Mouse	286	KKLGKSD-----	293
Rat	286	KKLGKSE-----	293
Yeast	294	ERMKGRANNAQKKTN	309

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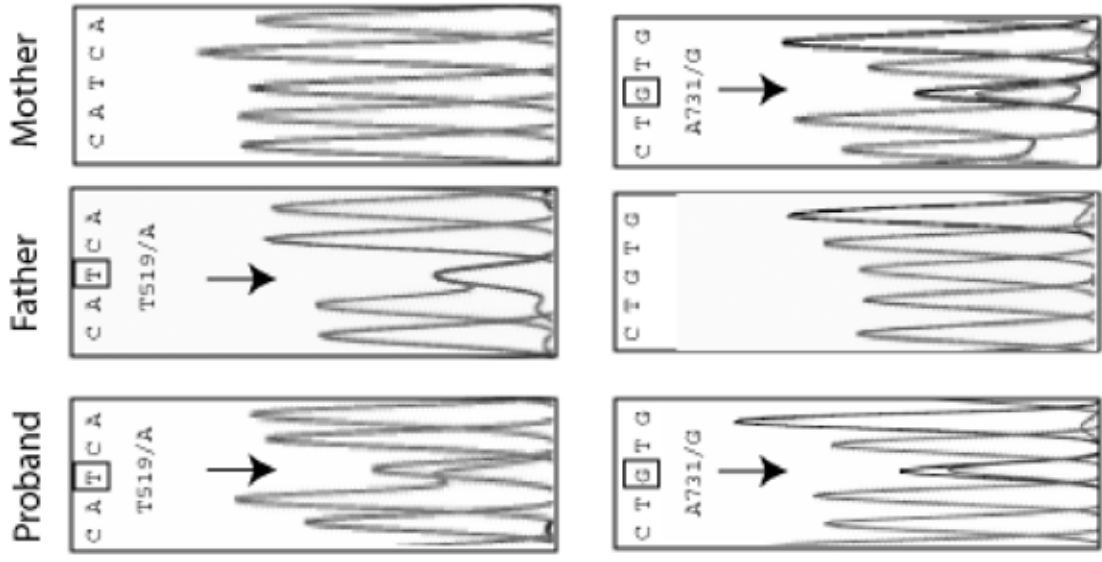


Figure 4. He et al., 2008

