Complete human CD1a deficiency on Langerhans cells due to a rare point mutation in the coding sequence

To the Editor:

The family of CD1 molecules is structurally similar to MHC class I molecules, but the 2 protein families mediate fundamentally different immune functions. MHC class I molecules present peptides to T cells, whereas CD1 molecules present lipids to natural killer T cells and other CD1-restricted T cells.1 CD1a is highly expressed on human Langerhans cells (LCs), a specialized mononuclear phagocyte that is prevalent in the epithelial cell layer of the skin and mucosal surfaces. Epidermal LCs can function as classical antigen-presenting cells (APCs) to induce naive T-cell responses in draining lymph nodes, but also have a regulatory function in the skin via local induction of regulatory T cells and maintenance of epithelial barrier integrity.2-3 Human dermal dendritic cells (DCs) also express CD1a, but in much lower amounts compared with LCs. CD1a+ dermal DCs, which coexpress CD1c, have been shown to efficiently stimulate CD4+ and CD8+ T cells in vitro.4-6 However, immune deficiencies due to selective CD1a defects have not been previously described, and it has proved difficult to dissect the specific role of CD1a in immune regulation.

During the course of a clinical study that involved analysis of APC subsets in human skin biopsies by flow cytometry, we identified a healthy Vietnamese individual, donor 007, who showed complete absence of CD1a expression on skin APCs (Fig 1, A). This case presented an opportunity to study the biological significance of CD1a expression. To check whether LCs were absent altogether in donor 007, we obtained a second skin biopsy, separated the epidermal tissue from the underlying structures, and stained the epidermal tissues with antibodies binding to CD1a and to HLA-DR. Donor 007 LCs displayed intense HLA-DR staining with typical dendritic morphology, but CD1a staining was minimal (Fig 1, B).

We next addressed whether the CD1a deficiency represented a generic expression defect, using monocyte-derived dendritic cells (moDCs) as a model. In keeping with our earlier observations, moDCs from donor 007 showed no surface CD1a expression by flow cytometry or immunohistochemistry (see Fig E1, A, in this article’s Online Repository at www.jacionline.org, in contrast to moDCs derived from a normal healthy control donor. Staining with other anti-human CD1a clones, OKT6 and NA1/34-HLK, showed the same result as staining with clone HI149 (see Figs E2 and E3 in this article’s Online Repository at www.jacionline.org). In addition, no costain with early endosome antigen-1 and CD1a was observed, excluding CD1a accumulation in early endosomes in donor 007 (Fig E1, B).

To address whether the CD1a defect was caused by a mutation in the CD1a gene, we invited the parents and all 4 siblings of donor 007 for a clinical assessment and CD1a expression analysis. Summary clinical information for the family members is presented in Table E1 in this article’s Online Repository at www.jacionline.org. Apart from donor 007’s father, who had severe Parkinson’s disease, the family members were generally healthy and displayed apparently normal skin barrier function and wound healing.

Both parents (001 and 002) and siblings 003, 004, and 006 showed normal CD1a surface expression on skin DCs and/or moDCs by immunohistochemistry and flow cytometry (Fig E4, A, in this article’s Online Repository at www.jacionline.org). However, skin DCs of sibling 005 showed complete absence of surface CD1a expression, similar to donor 007 (Fig E4, B). Blood DC subsets from family members, and from Singaporean healthy controls, were also analyzed by flow cytometry; the absence of CD1a had no impact on the development of blood DC subsets, and did not affect the expression of CD1c and CD1d molecules, excluding an intracellular CD1 protein trafficking defect (Fig E4, C-F).

To establish the genetic cause of the CD1a deficiency, we isolated RNA from moDCs for CD1a mRNA length and sequence analysis (see Fig E5, A, in this article’s Online Repository at www.jacionline.org). The lengths of the CD1a open reading frame from donor 007, from the parents, and from 1 sibling were identical, ruling out a shorter splice variant as the cause of the CD1a expression defect in donor 007. However, sequencing of the mRNA identified a single nucleotide polymorphism (SNP) (rs761269454) (Fig E5, B) that differed between donor 007 and nonaffected family members. The rs761269454 T to C conversion results in an amino acid change from Leucine to Proline at position 285 of the CD1a protein, located in the α3 domain of CD1a (Fig 2, A). Interestingly, parent 001 exhibited a double peak at this nucleotide position, suggesting that both the normal and mutant allele were expressed at the mRNA level, resulting in a normal CD1a phenotype at the protein level (Fig E5, B, and Fig E4, A).

We next isolated whole blood genomic DNA from all family members and sequenced the CD1a gene and 5000 bases upstream and downstream using Illumina MiSeq (see Fig E5, C [Sanger sequencing] and Table E2 [MiSeq] in this article’s Online Repository at www.jacionline.org). Donors 007 and 005 were heterozygous for rs761269454 (Fig E5, C, and Table E2), but expressed only the variant form of CD1a (Fig E5, B), in contrast to parent 001 and sibling 006, who were also heterozygous but expressed both alleles or at least the normal allele, respectively (Fig E4 and Fig E5, B). Intriguingly, we identified a second SNP rs538916791 that introduces a stop codon at amino acid 94 of the CD1a protein. The hereditary distribution of this SNP could explain the CD1a expression pattern: in the presence of the L285P SNP on one allele, the other allele was expressed normally. However, if one allele contained the L285P SNP and the other allele contained the stop codon SNP, for 005 and 007, only the L285P form could be expressed. To test whether the L285P mutation was sufficient to abrogate surface CD1a expression, we recombinantly expressed both the reference/wild-type and the mutant forms of CD1a in human embryonic kidney cells (a fibroblast cell line) and K562 cells (a granulocytic/monocytic cell line) (Fig 2, B). We chose 2 cell lines to address potential cell-type–specific differences in expression. Flow cytometry analysis showed that only the reference but not the mutant form of CD1a was expressed on the cell surface (Fig 2, B), whereas both forms were transcribed equally (Fig E3). Immunohistochemistry of transfected HEK cells confirmed this.

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finding (Fig 2, C). Different transfection ratios of normal to mutant CD1a resulted in the expected expression level of normal CD1a and excluded competition at the translational level (Fig 2, B).

In summary, we describe complete CD1a deficiency in 2 apparently healthy Vietnamese adults, and have identified a novel mutation responsible for the expression defect. This did not result in any apparent CD1a-related skin abnormalities, or in systemic immune impairment in either individual.

CD1a-restricted T cells specific for the mycobacterial lipopeptide didehydroxymycobactin can be detected in the blood of tuberculin-positive individuals ex vivo. Besides a potential role of CD1a-restricted T cells in antibacterial responses, presentation of natural skin lipids to CD1a-autoreactive T cells has been suggested to be essential for maintenance of the skin immune barrier. According to this hypothesis, a skin injury causes CD1a-expressing epidermal LCs to activate dermal CD1a-restricted T cells, resulting in IL-22 secretion, which, in turn, helps to repair any epithelial damage. Moreover, the inflammation caused by bee and wasp venom is mediated via CD1a-restricted self-reactive T cells in the skin. These venoms contain phospholipase A2,
which processes skin lipids that are then presented as neoantigens on CD1a, resulting in the activation of CD1a-restricted T cells.\(^8\)

None of the family members described here had a history of tuberculosis, although all are likely to have been exposed because tuberculosis is endemic in the region. Similarly, there was no apparent difference in the occurrence of common skin infections, or in wound healing, between family members displaying different CD1a expression patterns, and no family members recalled unusual reactions to bee or wasp stings.

These findings suggest that it is unlikely that CD1a surface expression is an essential element in the proposed pathway by which LCs are thought to function to maintain the integrity of the skin immune barrier.

**Daniela Cerny, PhD\(^{a,b}\)**

**Duyen Huynh Thi Le, MS\(^c\)**

**Trung Dinh The, MD, PhD\(^d\)**

**Roland Zuest, PhD\(^a\)**

**Srinivasan KG, PhD\(^a\)**

**Sumathy Velumani, MS\(^a\)**

**Chiea Chuen Khor, MD, PhD\(^d\)**

**Lucia Mori, PhD\(^{a,e}\)**

**Cameron P. Simmons, PhD\(^{a,e}\)**

**FIG 2.** Recombinant expression of the mutant CD1a-L285P reproduces the *in vivo* expression defect. A, Structure of the CD1a molecule in complex with a sulfatide (Protein Data Bank 1ONQ). The α domains (pink), β2 microglobulin (blue), and the position of L285P in the α3 subunit (green) are shown. B, CD1a surface expression of WT and L285P CD1a-transfected HEK and K562 cells analyzed by flow cytometry 24 hours after transfection. Cells were transfected with the indicated ratios of WT and L285P CD1a plasmid. Bar graphs show means ± SEM of % CD1a-expressing cells measured in 2 independent experiments with total \( n = 4 \). C, CD1a expression on transfected HEK cells analyzed by immunofluorescence microscopy. HEK, Human embryonic kidney; SSC-A, side scatter-area; WT, wild-type.
Severe allergic reaction onset

OR

Data presented as n (%). Bivalirudin was the reference group in the calculation of OR.

Allergic reaction onset

Anaphylaxis 1 (0.06) 10 (0.62) 11 (0.34) 10.0 (1.3-78.5)

Severe allergic reaction 4 (0.25) 18 (1.12) 22 (0.69) 4.5 (1.5-13.4)

Serious allergic reaction 1 (0.06) 10 (0.62) 11 (0.34) 10.0 (1.3-78.5)

Any allergic reaction 10 (0.62) 24 (1.5) 34 (1.06) 2.4 (1.2-5.1)

Bivalirudin (n = 1601) Pegnivacogin (n = 1605) Total (N = 3206) OR (95% CI)

Any allergic reaction 10 (0.62) 24 (1.5) 34 (1.06) 2.4 (1.2-5.1)

Serious allergic reaction 1 (0.06) 10 (0.62) 11 (0.34) 10.0 (1.3-78.5)

Severe allergic reaction 4 (0.25) 18 (1.12) 22 (0.69) 4.5 (1.5-13.4)

Nonsevere allergic reaction 6 (0.37) 6 (0.37) 12 (0.37) 1.0 (0.3-3.1)

Anaphylaxis 1 (0.06) 10 (0.62) 11 (0.34) 10.0 (1.3-78.5)

Allergic reaction onset < 1 h after study drug dosing 2 (0.12) 16 (1.0) 18 (0.56) 8.1 (1.9-35.1)

Severe allergic reaction onset < 1 h after study drug dosing 1 (0.06) 12 (0.74) 13 (0.40) 12.1 (1.6-92.8)

Data presented as n (%). Bivalirudin was the reference group in the calculation of OR.

OR, Odds ratio.
METHODS
Clinical methods
To study the index case and his family members, ethical approval was obtained from the Ethical Committee of the Hospital for Tropical Diseases of Ho Chi Minh City and the Oxford Tropical Research Ethics Committee. Following written informed consent, a detailed clinical assessment was performed by a single physician, and a blood sample was obtained for a full hematologic/biochemistry panel together with a sample for the immunological studies described below. Four of 7 participants also consented to a shave skin biopsy performed by the same physician.

Control samples consisted of anonymized blood specimens provided by healthy donors to the National University Hospital of Singapore Blood Bank. All donors gave written informed consent.

Skin biopsies
Shave biopsies were taken under local lignocaine anesthesia using DermaBlades (Personna Medical, AccuTec Blades Inc, Verona, Va). Biopsies were collected in RPMI medium and cut into 2 parts. One part was fixed overnight at 4°C in PBS containing 30% sucrose and 2% paraformaldehyde, then washed in 30% sucrose for 2 hours, and kept in PBS at 4°C until use. The other section of the biopsy was cut into small pieces and digested overnight at 37°C in RPMI medium containing 0.8 mg/mL collagenase (Type IV, Worthington-Biochemical, Lakewood, NJ) and 100 U/mL DNase (Roche, Ho Chi Minh City, Vietnam). Digested tissue was disrupted by manual pipetting, and connective tissue and debris were removed by filtering the cells through a 70-μm filter. Cells were labeled with the following antibodies: HLA-DR-PE-Cy7 (clone L243), CD45-V500 (clone HI30), CD1a-APC (clone HI149), and CD14-PE (clone M5E2) (all from Becton Dickinson, BD Biosciences, San Jose, Calif) before flow cytometry analysis. The gating strategy has been described elsewhere.11

Differentiation of monocyte-derived DCs, histology, and flow cytometry
PBMCs were isolated by Ficoll density gradient (GE Healthcare Life Science, Singapore) and frozen in liquid nitrogen for later analysis. Monocytes were isolated using CD14-microbead positive selection (STEMCELL Technologies Canada Inc, Singapore). CD14+ monocytes were cultured in RPMI medium supplemented with 10% FCS with 50 ng/mL recombinant human GM-CSF and 10 ng/mL IL-4 (both from Immuno Tools, Friesoythe, Germany) for 6 to 7 days to generate MoDCs. Anti-CD1a clone HI149 (Biomarkers and Immunoassays, Biologend, San Diego, Calif) or clone OKT6E2 was used for flow cytometry and histology.

For microscopy analysis, cells were seeded on Poly-L-Lysine–coated chamber slides (Ibidi, Planegg/Martinsried, Germany) and fixed with 4% paraformaldehyde for 20 minutes. Cells were then permeabilized with 0.1% Triton X-100 and blocked with 3% BSA for 2 hours before the antibody was added at room temperature and incubated for 2 hours. Hoechst (200 ng/mL; Invitrogen, Thermo Fisher Scientific, Carlsbad, Calif) was added for 5 minutes and slides were washed 3 times before addition of Prolong Gold (Life Technologies Corporation, Thermo Fisher Scientific, Carlsbad, Calif). Images were taken on an Olympus IX81 confocal microscope.

PBMC flow cytometry analysis
PBMCs were isolated using Ficoll (GE Healthcare) or cell preparation tubes (Becton Dickinson) and frozen for later analysis. Thawed cells were stained and analyzed using a FACS-LSRII (Becton Dickinson). For the DC subset analysis, the negative fractions of the CD14-positive sort (see moDC generation) were used. DC subsets were determined following a previously described gating strategy9 that involved using antibodies binding to lineage (CD19 [clone HIB-19], CD20 [clone 2H7], CD3 [clone UCHT1], CD56 [clone MEM188], CD45 [clone H30), HLA-DR (clone L243), CD14 (clone RM052; Life Sciences and Diagnostics, Beckman Coulter, Brea, Calif), CD11c (clone B-ly9), CD1c (clone L161), CD141 (clone AD5-14H12; MACS FlowCytometry, Miltenyi Biotec, Singapore), CD34 (clone 563; BD Pharmingen, BD Biosciences, San Jose, Calif), CD123 (clone 7G3), and Hoechst. For the analysis of B cells and the analysis of CD1d expression, PBMCs were stained with CD45 (clone H30), CD19 (clone HIB19; BD Pharmingen), CD1c (clone L161), CD1d (clone 51.1), CD3 (clone UCHT1), CD14 (clone RM052; Beckman Coulter), and Hoechst. All antibodies were purchased from Biolegend, unless otherwise stated.

Sequencing of messenger RNA and genomic DNA
For mRNA sequencing, moDCs were collected into catch buffer (10 mM Tris, pH 8, RNAase inhibitor RNAsin [%Promega Corporation, Madison, Wis]) and frozen at −80°C until use. cDNA was generated and amplified using the One-Step Pico Kit (Qiagen, Hilden, Germany) with the following primers: 5’-CTA CTT CCA TGT TTA GCT GCT TCC CC and 5’-TGT CTT CAC AGA AAC AGC GTT TCC T. The PCR product was loaded on an agarose gel and the product was isolated with a gel extraction kit (Qiagen).

Genomic DNA was isolated from whole blood using the DNeasy Blood & Tissue Kit (Qiagen). Sequencing of the CD1a gene was first performed using overlapping primers and sequencing the PCR products with Sanger sequencing (Fig E5, C).

For a deeper coverage, we used Illumina MiSeq to sequence the CD1a gene and approximately 5-kb region upstream and downstream of the coding regions, inclusive of the 5’ and 3’ UTRs. The entire 13.8-kb genomic sequences (>hg38.refGene_NM_001763 range=chr1:158294137-158262944) were subdivided into 5 shorter regions and were amplified for library preparation. Primer sequences and their positions can be found in the table below (Table E3).

Amplification was performed using 10 ng of total genomic DNA prepared from blood as template using LongAmp Taq DNA polymerase (New England Biolabs, Singapore) according to manufacturer’s instructions. PCR cycling conditions for amplicon regions 1, 3, 4, and 5 were 94°C for 30 seconds (initial denaturation), followed by 25 cycles of 94°C for 15 seconds (denaturation), 58°C for 30 seconds (annealing), and 65°C for 30 seconds (extension), with a final elongation of 65°C for 10 minutes. For amplifying the amplicon 2 region, the cycling profile was similar to above except that the annealing was carried out at a higher temperature (60°C for 30 seconds). Amplified products were purified using Agencourt Ampure XP beads (Beckman Coulter). Equimolar amounts of PCR products were pooled together for each sample separately for library preparation.

Libraries of pooled amplicons for each sample were prepared using the Nextera XT kit (Illumina, San Diego, Calif) according to manufacturer’s instructions. Libraries were constructed using 0.8 ng of pooled amplicons as starting material. Briefly, fragmentation of template DNA (5 μL) was carried out in 10 μL of Tagment DNA buffer using 5 μL of Amplicon Tagment Mix. Indexes were added to Tagmented DNA using 12 PCR cycles. The amplified and indexed libraries were purified and size selected using Agencourt Ampure XP beads (Beckman Coulter). The length one of the libraries was monitored using DNA 100 kits on the Agilent 2100 Bioanalyzer (Agilent Technologies, Singapore). Equimolar amounts of purified libraries were pooled and sequenced using indexed PE sequencing runs of 2 × 250bp on an Illumina MiSeq Personal Sequencer (MiSeq Control Software Version 2.4.1.3). Each individual sample was sequenced at an average depth of 2.3 million reads to detect SNPs in the genomic region of interest.

Data analysis
MiSeq reads were mapped to the HG38 reference genome with bowtie 2.24 SNP calls were made with Samtools Mpileup and BCFtools.25 Annotation of rs numbers was performed with an in-house custom script and data from dbSNP for the targeted region. SNP function was determined with snpEff and the Gencode V24 annotation.26,27 The data from this study are available in the NCBI BioProject database (ID: 315777).

Database screen for L285P prevalence
A comprehensive genetic database comprising more than 2000 Vietnamese individuals genotyped with the Illumina Human Exome array47,48 was screened but did not reveal the CD1A 285L location to be polymorphic. Further examination of exome-sequenced East Asian samples,29 as well as the 1000 Genomes project Phase 3 cosmopolitan database,30 also did not reveal any polymorphisms in the CD1 285L genomic location.
Generation of L285P mutant and expression in HEK and K562 cells

Construction of a BCMGSneo CD1a-expressing plasmid has been described elsewhere. To introduce the L285P mutant, an overlap PCR was performed using primer pair CD1a_XhoI_FOR 5'-CTTCTCGAGATGCTGTTTTGCTACTTCC-3' and CD1a_mut_REV 5'-ATGTCTCTGGCCTCCTGGACTGTGTGCCTAC-3' and primer pair CD1a_NotI_REV 5'-CCACACGCGGCGCCTAAACAGAAACAGCGTTTC-3' and CD1a_mut_FOR 5'-TGAAGCAACAGCATCCAGAGGGCCAGGA CATC-3'. Products were purified, mixed, and used as a template for an overlap PCR using primer pair CD1a_XhoI_FOR and CD1a_NotI_REV. The final product was digested with XhoI and NotI and subsequently ligated into the parental vector.

References

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E2. Vincent MS, Xiong X, Grant EP, Peng W, Brenner MB, CD1a-, b-, and c-restricted TCRs recognize both self and foreign antigens. J Immunol 2005;175:6344-51.
FIG E1. CD1a deficiency is generic and not confined to primary skin APCs. **A**, moDCs from a healthy control donor and from donor 007 were stained either on the surface or intracellularly (i.c.) with anti-CD1a and analyzed by flow cytometry. **B**, Immunohistochemistry of moDCs stained with anti-CD1a (green), anti-EEA, an early endosome marker (red), and Hoechst (blue). Healthy control data are representative of 2 individuals. EEA, Early endosome antigen; FSC-A, Forward scatter-area; SSC-A, side scatter-area.
FIG E2. Absence of surface CD1a detection in donor 007 is independent of the antibody clone used. Monocyte-derived DCs from donors 003 and 007 were stained with anti-human CD1a clone OKT6. A positive signal was observed only for donor 003. FSC-A, Forward scatter-area; SSC-A, side scatter-area.
FIG E3. CD1a L285P is transcribed but not expressed as a protein on the cell surface. **A**, mRNA expression of HEK and K562 cells-transfected WT and mutant forms of CD1a. **B**, Detection of WT and mutant CD1a by anti-CD1a clone NA1/34-HLK. HEK and K562 cells were transfected for 72 hours before analyzing the surface expression of CD1a by flow cytometry. The expression pattern is similar to the one detected by anti-CD1a clone HI149 (Fig 2). HEK, Human embryonic kidney; SSC-A, side scatter-area; WT, wild-type.
FIG E4. CD1a deficiency is selective and spares CD1c and CD1d expression. CD1a expression on moDCs from affected individuals and family members analyzed by flow cytometry (A) and immunofluorescence microscopy (B). C, Frequency of peripheral blood myeloid DC subsets, pDCs, and CD34+ stem cells in the affected family (black symbols) and healthy controls (open symbols). D, Frequency of total B cells and naive B cells (CD19+ IgD−) in 007 family members and control donors. CD1c (E) and CD1d expression (F) on blood DCs from 007 family members and control donors. Representative histograms for 007 and 1 control donor are shown. Donor 007 (with the CD1a defect) is indicated in red. Cells from donor 005 were not available for this analysis. MFI, Median fluorescence intensity; ns, nonsignificant.
FIG E5. Sequence analysis of CD1a mRNA and gDNA identifies a novel mutation causing CD1a deficiency. 

**A,** mRNA length of the CD1a ORF amplified from mRNA extracted from moDCs generated from 4 family members.

**B,** A thymidine (T) to cytidine (C) mutation identified by mRNA sequencing of donor 007 and family members is indicated with a red box.

**C,** Sequences of the genomic DNA (gDNA) of family members; the CD1a gene position coding for the mutated mRNA is indicated with a red box.

**D,** CD1a genotype tree of the family members. The family members with CD1a deficiency are indicated in red. gDNA, Genomic DNA; ORF, Open Reading Frame.
| Subject | 001 | 002 | 003 | 004 | 005 | 006 | 007—index case* |
|---------|-----|-----|-----|-----|-----|-----|-----------------|
| CD1a status | rs538916791: C/C | rs538916791: C/A | rs538916791: C/A | rs538916791: C/A | rs538916791: C/A | rs538916791: C/A | rs538916791: C/A |
| rs761269454: T/C | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a |
| rs761269454: T/T | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a | Normal CD1a |
| Sex | Female | Male | Male | Male | Male | Male | Male |
| Year of birth | 1959 | 1956 | 1991 | 1982 | 1986 | 1985 | 1989 |
| Occupation | Farmer | Farmer | Businessman | Businessman | Construction worker | Computer repair business | Medical doctor |
| History (general) | Arthritis, hypertension, vertigo | Parkinson’s disease, anal fistula | Unremarkable | Dengue, allergic rhinitis | Gastritis, sinusitis, Bell’s palsy | Labyrinthitis | Complex forearm fracture, internal fixation |
| Skin abscesses or recurrent skin infections? | Occasional, in childhood only | Occasional, in childhood only | No | No | Moderate frequency, in childhood only | Moderate frequency, in childhood only | Moderate frequency, in childhood only |
| Other skin disorders | Mild acne in the past | Moderate acne in the past | Mild acne (ongoing) | Mild acne and an episode of herpes zoster in the past | Mild acne and folliculitis (ongoing) | Moderate acne (ongoing) | Mild acne and fungal skin infections in the past |
| Wound healing | Normal, a few days | Normal, a few days | Normal, a few days | Normal, a few days | Normal, a few days | Normal, a few days | Normal, a few days |
| Reactions to bee stings | No specific event recalled | No specific event recalled | 1-2 events, mild reactions | 1-2 events, mild reactions | 10 events, some multiple; mild reactions | >20 events, some multiple; moderate reactions | 1-2 events, mild reactions |
| Reactions to wasp stings | No stings | No stings | No stings | 1-2 events, moderate reactions | Once, moderate reaction | 1-2 events, moderate reactions | No stings |
| Examination findings | General examination | Parkinsonian | Unremarkable | Unremarkable | Unremarkable | Unremarkable | Unremarkable |
| BMI | 24.6 | 20.8 | 20.3 | 21.5 | 21.8 | 19.6 | 20.3 |
| Visible BCG scar† | No | No | Yes, normal appearance | No | No | No | Yes, normal appearance |
| Mantoux (5IU PPD-S) | Not done | Not done | 9-mm response | Not done | 3-mm response | Not done | 10-mm response |
| Laboratory results | White blood cells | 6.57 | 9.12 | 4.76 | 5.31 | 5.6 | 4.47 | 10.2 |
| Hemoglobin | 14 | 15.8 | 17.3 | 16 | 15.9 | 16.7 | 17 |
| Platelets | 222 | 241 | 161 | 174 | 227 | 127 | 216 |
| Glucose | 4.2 | 4.4 | 5.1 | 5.1 | 4.8 | 4.8 | 5.6 |
| Creatinine | 74 | 85 | 75 | 80 | 94 | 95 | 76 |
| AST | 31 | 22 | 16 | 18 | 23 | 26 | 20 |
| ALT | 21 | 11 | 18 | 28 | 19 | 38 | 40 |
| GGT | 8 | 31 | 23 | 23 | 24 | 29 | 26 |
| Cholesterol | 4.4 | 5.1 | 4.4 | 4.8 | 4.2 | 4.4 | 4.7 |
| HDL | 0.9 | 1.2 | 1.3 | 1.2 | 0.8/0.9 | 1.3 | 1.3 |
| LDL | 2.8 | 3.4 | 2.9 | 3.4 | 2.9 | 2.8 | 3.2 |
| Triglyceride | 1.7 | 1 | 1.4 | 1.5 | 2.5/2.6 | 1.1 | 1.3/1.6 |

Local normal ranges for hematology and biochemistry tests (abnormal results indicated in boldface):

- White blood cells: Male/female: 6-10 K/mL.
- Hemoglobin: 14.5-15.7 g/dL for men and 13-14 g/dL for women.
- Platelets: 201-324 K/mL for men and 211-337 K/mL for women.
- Glucose: 3.9-6.4 mmol/L.
- Creatinine: 62-120 µmol/L for men and 53-100 µmol/L for women.
- AST (aspartate aminotransferase): 0-40 U/L for men and 0-37 U/L for women.
- ALT (alanine aminotransferase): 0-40 U/L for men and 0-33 U/L for women.
- GGT (gamma glutamyl transferase): 11-50 U/L for men and 7-32 U/L for women.
- Cholesterol: 3.9-5.2 mmol/L.
- HDL (high density lipoprotein): 0.9-1.78 mmol/L.
- LDL (low density lipoprotein): 1.15-3.4 mmol/L.
- Triglyceride: 0.46-1.6 mmol/L for men and 0.68-1.88 mmol/L for women.

*Additional investigations were performed on the index case (007): electrolytes and plasma protein levels, bone biochemistry, thyroid function tests, immunoglobulin A, M, & G levels, and an abdominal ultrasound were all normal.

†BCG vaccination status uncertain—only subjects 003, 005, and 007 are thought to have received any vaccinations in infancy, but there are no records of what they were given.
## TABLE E2. SNP calls for the 13,807 base pairs sequenced in this study

| SNP ID  | Reference name | Position   | snpEff call       | Reference base | Alternative base | Donor 001 refC | Donor 001 altC | Donor 001 totC | Donor 002 refC | Donor 002 altC | Donor 002 totC | Donor 003 |
|---------|----------------|------------|-------------------|----------------|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|-----------|
| rs75981383 | chr1         | 158249693  | Upstream modifier | C             | G               | Het            | 39.20%         | 40.76%         | 2495          | Het            |               |           |
| rs3181029  | chr1         | 158250175  | Upstream modifier | C             | T               | Het            | 40.09%         | 43.90%         | 4647          | Het            |               |           |
| rs3181031  | chr1         | 158250679  | Upstream modifier | T             | C               | Het            | 42.39%         | 40.72%         | 2699          |               |               |           |
| rs858998   | chr1         | 158250785  | Upstream modifier | C             | T               | Hom            | 0.32%          | 84.07%         | 5890          | Het            |               |           |
| rs76519430 | chr1         | 158251080  | Upstream modifier | T             | C               | Hom            | 0.10%          | 81.32%         | 4165          | Hom            |               |           |

**Note that ~20% of the reads were of low quality and not included in the SNP calls after mapping. SNPs in boldface are discussed in the text. altC, Alternative base; _altC, percent of total reads with alternative base; het, heterogeneous; homo, homogeneous; refC, reference base; _refC, percent of total reads with reference base; totC, total number of reads spanning the position.**
TABLE E2. (Continued)

| Donor | 003 refC | 003 altC | 003 totC | 004 refC | 004 altC | 004 totC | 005 refC | 005 altC | 005 totC | 006 refC | 006 altC | 006 totC | 007 refC | 007 altC | 007 totC |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 41.68% | 39.89% | 3397 Het | 41.65% | 40.11% | 3712 Het | 41.14% | 37.41% | 2144 Het | 40.23% | 41.98% | 3435 | 39.30% | 37.89% | 3112 |
| 42.31% | 40.94% | 6209 Het | 41.97% | 42.46% | 6562 Het | 40.82% | 41.98% | 3435 | 39.30% | 37.89% | 3112 |
| 41.59% | 43.19% | 3200 Het | 42.90% | 41.55% | 3427 Het | 39.49% | 42.36% | 1636 Het | 41.14% | 37.41% | 2144 Het | 40.23% | 41.98% | 3435 |
| 40.19% | 82.93% | 2126 Hom | 0.05% | 83.99% | 2130 Hom | 0.19% | 80.97% | 1035 Het | 40.46% | 38.47% | 5045 |
| 36.82% | 43.47% | 4150 Het | 37.44% | 41.71% | 4725 Het | 33.22% | 42.75% | 3064 Het | 40.37% | 45.65% | 2668 Het | 34.64% | 50.41% | 3271 |
| 38.85% | 47.87% | 4903 Het | 40.27% | 45.80% | 5419 Het | 36.44% | 48.09% | 3271 Het | 43.04% | 51.58% | 2935 Het | 38.95% | 45.51% | 3271 |

| Donor | 003 refC | 003 altC | 003 totC | 004 refC | 004 altC | 004 totC | 005 refC | 005 altC | 005 totC | 006 refC | 006 altC | 006 totC | 007 refC | 007 altC | 007 totC |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 42.17% | 42.41% | 2511 Het | 42.91% | 40.50% | 2736 Het | 39.91% | 43.04% | 1601 Het | 39.86% | 44.05% | 1814 |
| 41.70% | 47.23% | 5461 Het | 41.89% | 47.31% | 5842 Het | 41.77% | 44.78% | 3546 Het | 40.15% | 45.06% | 2730 |
| 38.59% | 38.09% | 7165 Het | 38.79% | 36.77% | 6992 Het | 40.59% | 36.08% | 3930 Het | 43.53% | 45.06% | 7574 |
| 41.72% | 41.53% | 4293 Het | 42.66% | 41.76% | 4440 Het | 37.10% | 42.54% | 5251 Het | 38.80% | 40.59% | 5943 |
| 44.43% | 43.77% | 6815 Het | 44.31% | 42.80% | 7260 Het | 43.43% | 41.75% | 4156 |

**TABLE E2.** (Continued)
| Genomic coordinates   | Target      | Forward       | Reverse                  | Amplicon size | Overlap with next amplicon |
|-----------------------|-------------|---------------|--------------------------|---------------|---------------------------|
| Chr 1: 158249137-158252175 | Amplicon-1  | atcaaacctaagctgactcctc | accagacccatctctctattg     | 3039          | 479                       |
| Chr 1: 158251697-1582555283 | Amplicon-2  | aagtgttcctgcctttcttccag | taatgtttccagtttccac        | 3587          | 307                       |
| Chr 1: 158254976-158257976 | Amplicon-3  | atggatccctctctctctgattc | ttaaatgggacatgggagga        | 3001          | 224                       |
| Chr 1: 158257752-158261027 | Amplicon-4  | ggtccagacacacactgaacac  | acaggtcagatctaattgtg        | 3276          | 488                       |
| Chr 1: 158260359-158262943 | Amplicon-5  | aagtgatctaagaagcttaagtg | agttgttcacatgttaattgc       | 2404          | NA                        |

NA. Not applicable/available.