Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Renner ED, Torgerson TR, Rylaarsdam S, et al. *STAT3* mutation in the original patient with Job's syndrome. N Engl J Med 2007;357:1667-8.

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Second Job Syndrome patient

A second female patient was described in the initial report of Job syndrome (case #2)[1]. This patient died of chronic lung disease at age 19. She had no offspring and was the only affected member in her family which has been lost to follow up.

Methods

Mutation Analysis

Mutation analysis was performed on cDNA and confirmed by genomic DNA sequencing for members of this cohort. To prepare cDNA, total mRNA was extracted from $5x10^6$ fresh peripheral blood mononuclear cells (PBMC) using Trizol reagent (Invitrogen, Carlsbad, CA) and subjected to first strand cDNA synthesis using the Omniscript RT Kit (Qiagen, Valencia, CA) per the manufacturer's protocols. Genomic DNA was prepared from buffy coats of heparinized blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. The *STAT3* gene was amplified from cDNA or gDNA using specific oligonucleotide primers and polymerase chain reaction. The amplified gene fragments were sequenced using the ABI Big Dye Terminator mix (Applied Biosystem, Foster City, CA), and analyzed with a 3730xl DNA Analyzer (Applied Biosystem, Foster City, CA). Mutations were reported using the nomenclature of den Dunnen and Antonarakis[2].

Oligonucleotide primer sequences

The STAT3 cDNA was amplified and sequenced in two fragments using the following primer sets: F 5'-AACCGGATCCTGGACAGGCA-3' / R 5'-AGCTCCTCAGTCACAATCAG-3' and F 5'-GGAAGAATCCAACAACGGCA-3' / R 5'-GGAGGCACTTGTCTAAGAAC-3'. Exon 12 of the *STAT3* gene was amplified and sequenced from gDNA using the following primer set: F 5'-GGACGTTGCAGCTCTCAGAGG-3' / R 5'-CTGCAGAGAGGCTGCCGTTG-3'.

References

1. Davis SD, Schaller J, Wedgwood RJ. Job's Syndrome. Recurrent, "cold", staphylococcal abscesses. Lancet 1966;1:1013-5.
2. den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. Hum Genet 2001;109:121-124.