Somatic mutations in CD8+ cells in multiple sclerosis patients and controls

Miko Valori[1], Lilja Jansson[1], Pekka Ellonen[2], Satu Mustjoki[3,4], Pentti J. Tienari[1] (presenting author)

[1] Translational Immunology, Research Programs Unit, University of Helsinki, [2]Neurocenter, Helsinki University Hospital, Helsinki, Finland. [3]Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, [4] Hematology Research Unit Helsinki, University of Helsinki and Helsinki University Hospital Comprehensive Cancer Center.

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

HUS
Helsinki University Hospital

Abstract

Background: Somatic mutations have a central role in cancer but their role in other diseases such as autoimmune disorders remains unclear . Although cancer risk is not increased in MS patients, there are multiple shared risk factors in MS and cancers (most notable Epstein-Barr virus).

Aims: To analyze the frequency of somatic mutations and possible mutational hot-spots in blood CD8+ cells in relapsing MS patients and age- and sex-matched controls.

Methods: 21 newly-diagnosed relapsing MS patients' and 21 controls' peripheral blood was fractionated into CD4+, CD8+, CD19+ cells. We performed deep next-generation sequencing of the CD8+ cells' DNA and control DNA targeting to coding regions of 2555 immune and cancer-related genes to detect somatic mutations.

Results: We discovered nonsynonymous somatic mutations in all MS patients and controls in the CD8+ fraction. The total number of amino-acid changing mutations was similar in the MS and control group. The median number of mutations (n=4), and median allelic fraction (0.5%) were similar in the MS and control group. The number of mutated genes/mutations was 133/215 in patients and 150/251 in controls, 32% of the mutations were predictably deleterious in both groups. There were no obvious mutation hotspots, although a known activating STAT3*D661Y mutation, discovered in our pilot study, was now detected in a second MS patient. We therefore screened by amplicon sequencing the exons encoding the SH2 domain in additional MS patients and found that 9 (11%) of the 81 patients tested had somatic mutations in STAT3 in their CD8+ cells.

Conclusions: We have outlined an efficient approach for discovering low-frequency somatic mutations in blood cells. This powerful pipeline demonstrates that all MS patients and controls have somatic mutations in their CD8+ cells. These results define a rather individual landscape of somatic mutations in CD8+ cells in both MS patients and controls. The role of the mutant CD8+ clones (including those with STAT3 mutations) in MS is presently unclear, but their potential role warrants further research.

Introduction

In relapsing MS there is evidence based on genetics, environmental risk factors and treatment paradigms that leukocyte dysfunction plays a major role in the disease. Most MS plaques at autopsy are dominated by marcophages that phagocytose myelin debris (post-inflammatory event), whereas analysis of rare cases of acute plaques have identified clonal CD8+ T cells (1).

Somatic mutations in autoimmune disease. There are only few studies on leukocyte somatic mutations in autoimmune disease (2,3). Somatic mutations in the STAT3 gene in CD8+ T-cells have been found in 40% of patients with LGL leukemia, especially in those who presented with rheumatoid arthritis (4). Another recent study discovered somatic mutations in CD8+ cells in 20% (5/25) of patients with newly-diagnosed rheumatoid arthritis and in 5% (1/20) of controls (5).

Somatic mutations in MS. Indirect evidence of somatic mutations in autoreactive T cells has been previously reported in MS by using the HPRT assay (6,7). This assay screens mutations which impair the function of the X-chromosomal HPRT gene. Mutants are detected by positive selection (viable clones) using a toxic analogue, whose toxicity depends on the enzymatic activity of HPRT. Using next-generation sequencing of 986 cancer and immunity related genes and median coverage of ca. 700x, we have recently demonstrated directly the existence of blood leukocytes harbouring somatic mutations in 60% of MS patients (8). After a median follow-up time of 2.3 years, 96% of the mutations were still detectable indicating persistence of the mutant clones. Of the persistent mutations 88% were in CD8+ cells. Therefore, we have here first focused on CD8+ cells with an improved methodology (2555 genes with 2000x coverage).

Subjects and methods

Patients were recruited at the Helsinki University Hospital during their diagnostic examinations. This study has been approved by the regional ethics committee (Dno 83/13/03/01/2013). Mean age of the 21 MS patients was 35.2 yrs (75% females), mean age of the 21 controls was 35.2 yrs (75% females).

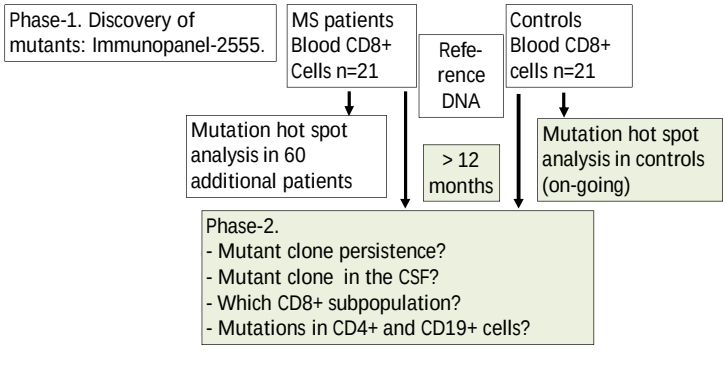
Cell separation. Peripheral blood mononuclear cells (PBMCs) were extracted from 140 ml venous EDTA blood using Ficoll-Paque PLUS (GE Healthcare) and positive selection with CD4, CD8 and CD19 antibody MicroBeads was performed (Miltenyi Biotec).

Target genes and sequencing. A gene panel that consists of 2555 genes related to immunity and cancer was designed for mutation screening. Target enrichment for all exons was carried out with the Nimblegen SeqCap exon capture system (Roche NimbleGen). Resulting library was sequenced with a HiSeq 2500 instrument (Illumina) using 150bp paired end reads.

Screening phase data analysis. Sequencing reads were mapped using BWA MEM and PCR duplicates were removed using Picard MarkDuplicates. VarScan2 with added custom logic was used for somatic mutation calling. Each sequenced cell population acted in turn as the VarScan2 "tumor" sample from which somatic mutations were called, and merged data from the reference DNA (from 8 health subjects) acted as the "normal" sample. Analyzer (M.V.) was blinded to the diagnoses (MS or control).

Figure 1. Flow-chart of the study.

(Phase-1 has been completed, phase-2 is in preparation)



Results

21 newly-diagnosed relapsing MS patients and 21 age- and sex-matched matched controls were analysed. Using the improved methodology we were able to discover high-confidence somatic non-synonymous mutations in all participants in their CD8+ cells. The number of mutations was similar in MS patients and controls (table). The number of mutations per participant (range 1-15) did not show any significant correlation with age (Figure 2).

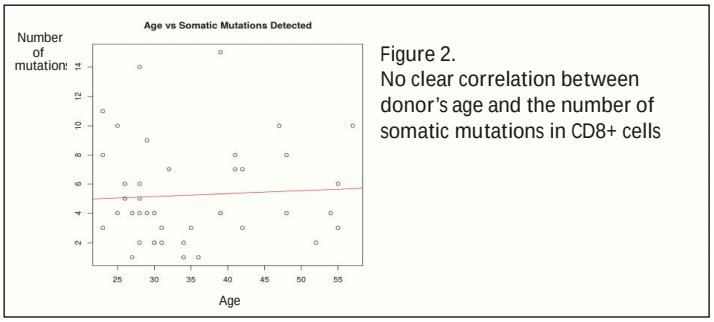


Figure 2.
No clear correlation between donor's age and the number of somatic mutations in CD8+ cells

The most frequently mutated genes were *STAT3* (6 mutations), *SMARCA2* (4 mutations), *DNMT3* (3 mutations).

A potentially interesting finding was the discovery of the known activating STAT3*D661Y (discovered in our pilot study) in a second MS patient. We therefore screen by PCR amplicon sequencing the exons 19-22 (encoding the SH2 domain) in additional MS patients' CD8+ cell's DNA. Altogether 9 (11%) of the 81 patients tested had high-confidence somatic mutations in *STAT3* (S614R in 4 patients, D661Y in two, Y640F, D661V, V671L each in one). We will perform the same analysis in controls and are currently collecting more CD8+ cells from controls.

Table. Summary data from Phase-1 (Discovery of somatic mutations)

	MS (n=21)	Controls (n=21)
Frequency of subjects with mutations in CD8+ cells (non-synonymous mutations)	100%	100%
Total number of mutations (high-confidence mutations)	215	251
Mutation number/subject	1-11 (median 4)	1-15 (median 4)
Mutational hot spots	Possible	No
Median allelic fraction	0.5% (range 0.1-12.7%)	0.5% (range 0.2-11.9%)
Number of mutated genes	133	150
Mutations with CADD >20*	32%	32%

*CADD scores of 20 or more denote predictably deleterious mutations.

Conclusions

→ With the new discovery pipeline somatic mutations can be found in all all participants in CD8+ cells. The frequency of mutations was similar in MS patients and controls.

→ The mutations were highly individual with no obvious hotspots.

→ A possible hotspot is the STAT3 SH2 domain (11% of patients). However, we have not yet analyzed representative numbers of controls to judge how specific this finding is to MS (STAT3 mutations may represent unspecific factors favoring clonal proliferation/survival).

→ Follow-up sampling is underway to analyze the frequency of the STAT3 SH2 domain mutations in controls, to characterize the mutant CD8 clones' phenotypes more in detail, their persistence in time, presence in the CSF and other blood cell types (CD4+ and CD19+).

→ Somatic mutations are ubiquitous in CD8+ cells and their role as drivers of autoimmunity warrants further study.

References: 1. Babbe H et al. Clonal expansions of CD8 (+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000; 192(3): 393-404. 2. Holzelova E *et al* (2004) Autoimmune lymphoproliferative syndrome with somatic fas mutations. *N Engl J Med* 351(14): 1409-1418. 3. Niemela JE *et al* (2011) Somatic KRAS mutations associated with a human nonmalignant syndrome of autoimmunity and abnormal leukocyte homeostasis. *Blood* 117(10): 2883-2886. 4. Koskela HL *et al* (2012) Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med* 366(20): 1905-1913. 5. Savola P et al. Somatic mutations in clonally expanded cytotoxic T lymphocytes in patients with newly diagnosed rheumatoid arthritis. *Nat Commun 2017;8:15869*. 6. Allegretta M et al (1990) T cells responsive to myelin basic protein in patients with multiple sclerosis. *Science* 247(4943): 718. 7. Sriram S (1994) Longitudinal study of frequency of HPRT mutant T cells in patients with multiple sclerosis. *Neurology* 44(2): 311-315. 8. Valori M et al. A novel class of somatic mutations in blood detected preferentially in CD8+ cells. *Clin Immunol 2017;175:75-81*.

Disclosure This study has been financially supported by research grants from the Helsinki University Hospital, University of Helsinki, the Multiple Sclerosis Foundation of Finland, the Finnish Cultural Foundation, Biogen Finland, Sanofi-Genzyme, Roche and Novartis. Pentti J. Tienari has received lecture fees or financing of congress trips from Bayer, Biogen, Merck, Novartis, Orion, Pfizer, Roche, Sanofi-Genzyme, Santen and Teva. Miko Valori, Lilja Jansson , Pekka Ellonen and Satu Mustjoki have nothing to declare.