Analysis of Lymphocyte and Clinical Profile among Non-monogenic Common Variable Immunodeficiency Patients with and without Class Switch Recombination Defect

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Common Variable Immunodeficiency (CVID) is the most common heterogeneous inborn errors of immunity (IEI) with reduced class-switched memory B cells (SMB), hypogammaglobulinemia, respiratory infections, autoimmunity, enteropathy and lymphoproliferation [1]. A group of monogenic defects are associated with the CVID phenotype [2, 3], although 50-80% of patients with no known IEI monogenic defect [4], lead to non-monogenic CVID. Class switch recombination (CSR) is a DNA rearrangement of the immunoglobulin (Ig) heavy-chain locus that changes Ig isotype from IgM to IgG, IgA and IgE, generating SMB or plasma cells [5]. Defect in genes involved in CSR will cause CSR-defect (CSR-D) leading to monogenic CVID, however among non-monogenic CVID patients, CSR-D has been surprisingly observed [6]. CSR-D is associated with dramatically reduced SMB levels and specific clinical manifestations in CVID patients [7]. We aimed to evaluate alterations in B and T lymphocyte subpopulations and clinical manifestations among genetically unsolved CVID patients with or without CSR-D to provide distinctive immunological and clinical phenotype of such CVID patients.

We included 30 genetically unsolved CVID patients in our study from the Iranian Immunodeficiency Registry Center. Using the whole-exome sequencing, we excluded

known IEI monogenic defects [8] in patients, as previously described [4, 9]. Based on clinical diagnosis [10], patients were classified into different groups of infection only (IO), autoimmunity (AI), chronic enteropathy (CE) and lymphoproliferation (LP). We also included 30 age- and sex-matched healthy controls (HC). The study was approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.CHMC.REC.1399.003) and written informed consent was obtained from each subject.

Based on serum Ig levels, we classified our patients into two groups, CSR-D and Non CSR-D. CVID patients with decreased IgG and IgA, but normal IgM levels were considered as CSR-D. The rest of the patients were categorized as Non CSR-D. The laboratory methods have been previously described [11]. In brief, extracellular and intracellular flow cytometry was conducted to detect all B and T cell subsets. IBM SPSS Statistics for Windows, version 24 (IBM Corp, USA) was used for conducting statistical analyses.

Among the CVID patients **(Table S1)**, 16.67% (5 out of 30) showed a defect in CSR. In CSR-D group IgM levels, delayed diagnosis and pneumonia were significantly higher **(Figure S1 and Table S2)**. Of note, all cases with bronchiectasis belonged to Non CSR-D group, suggesting the functional role of the residual IgM in CSR-D patients. Among CSR-D patients, 80% were IO and 20% were non-infectious phenotype (exclusively CE). In Non CSR-D population, we also observed IO as the most frequent clinical phenotype (56%) (**Table S2**).

All gating strategies for lymphocyte subsets have been previously presented [12]. We observed no statistically significant differences in lymphocyte subsets between CSR-D and Non CSR-D **(Figure S2)**. However, in comparison to HC, Non CSR-D group showed

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significant decreases in total, marginal zone (MZB) and IgM only memory B cells (IgM only MB), SMB, plasmablasts (PB), total, naive, central memory (CM) and regulatory (Treg) $CD4^+$ T cells, and naive $CD8^+$ T cells and also significant increases in total, effector memory (EM), terminally differentiated effector memory (T_{EMRA}), activated and cytotoxic $CD8^+$ T cells. The same reduction of SMB, IgM only MB and PB was observed for CSR-D compared to HC **(Table S3)**. Normalization of B cells and MZB was in line with higher level of IgM and phenotype of CSR-D. Based on the calculated cut-off values, we observed more percentage of patients with decreased SMB, IgM only MB, helper1 T cells (Th1), Treg and naive $CD8^+$ T cells in addition to a higher percentage of patients with increased $CD21^{low}$ and transitional (Tr) B cells, CM $CD4^+$ T cells and T_{EMRA} T cells among CSR-D than that of Non CSR-D **(Table S4)**.

Observation of CSR-D among CVID patients without known monogenic defects, suggests that defects in the other genes involved in CSR may have a potential role in the pathogenesis of CVID rather than the well-known genes. Some of these potential genes have been predicted to cause CVID [6]. Regarding detection of CSR-D among 17% of our non-monogenic CVID patients, we expected decreased SMB levels in CSR-D group compared to Non CSR-D. Nevertheless, this reduction was observed among 100% and 96% of CSR-D and Non CSR-D patients, respectively, with no statistically significant difference. It seems that CSR-D in non-monogenic CVID patients is too mild to cause significant alterations in SMB levels or result in other immunological and clinical features.

The cut-off values showed that defect in CSR had noticeable effects on some lymphocyte subsets, leading to a marked higher number of CSR-D patients having decreased memory B cells, Th1, Treg and naive $CD8^+$ T cells and increased $CD21^{low}$ B cells, Tr, $CD4^+$ T cells and T_{EMRA} T cells compared to Non CSR-D patients. Abnormalities in some of the above-

mentioned lymphocyte subsets regardless of CSR-D have been previously demonstrated to be associated with particular clinical phenotypes besides having a role in prediction of the CVID development for hypogammaglobulinemia children [12, 13]. Overall, it would strongly support the prominent role of abnormalities in $CD21^{low}$ B cells, Th1, Treg and naive and T_{EMRA} T cells in development of more severe features such as AI and CE among CSR-D patients, later in their lives.

We expected much more severity of clinical complications among CSR-D patients [14]. However, to our surprise, despite higher rate of infection, pneumonia and delayed diagnosis all patients suffering from bronchiectasis fell into the Non CSR-D group indicating the presence of immune dysregulation in the development of bronchiectasis in CVID patients. It seems that having no genetic defect in CVID related exomes may alleviate the adverse impacts of defects in CSR and lead to a milder form of the disease. In summary, we can conclude that observation of CSR-D among genetically unsolved CVID patients could be a result of defects in predictive genes, epigenetic changes and environmental or host-intrinsic factors leading to mild modification of B or T cell response pathways which in turn fails to cause severe clinical conditions or considerable alterations.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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