Genetic Analysis of Acid β-Glucosidase in Patients with Multiple Myeloma from Central Taiwan: A Small-Cohort Case-Control Study

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Multiple myeloma · Glucocerebrosidase · Acid β-glucosidase

Abstract
Introduction: Multiple myeloma (MM) is an incurable, biologically heterogeneous disease of the plasma cells, associated with older age and is more common in men. Gaucher disease, caused by mutation in acid β-glucosidase (glucocerebrosidase, GBA) gene, has been linked to multiple cancers, especially MM. Pathological accumulation of glucosylceramidase and complex glycosphingolipids coupled with chronic inflammation may be the cause of cancer in patients with Gaucher disease. In this study, we hypothesized patients with MM have mutations in the GBA gene and analyzed patients with MM to determine whether they have a higher frequency of GBA variants. Methods: Twenty-four MM samples were acquired from the Human Biobank, China Medical University Hospital, Taichung, Taiwan. GBA mutations were detected by polymerase chain reaction-directed DNA sequencing. Results: We found no mutations in the coding regions of GBA in any of the 24 study subjects. However, two single-nucleotide polymorphisms, rs2070679 and rs2361534, were identified. A significant difference was observed between the study and control groups (p = 0.0028) in rs2361534 allele distribution, with the C allele frequency being higher in patients (1/48, 2.1%) than in the control group (5/3030, 0.16%, Taiwan Biobank). Conclusion: In this study, the sample size was limited and GBA enzyme activity was not measured; therefore, we could not establish a direct correlation between MM and GBA mutations. However, the association of rs2361534 suggests that regions around this single-nucleotide polymorphism may be involved in MM. The relationship between MM and GBA mutations remains unclear. A large sample is required for a detailed analysis of this potential relationship.

Introduction
Multiple myeloma (MM) is an incurable, biologically heterogeneous disease of the plasma cells that is associated with older age and is more common in men [1]. It is the second most common hematological malignancy after lymphoma, with a global age-standardized incidence of 2.1 per 100,000 individuals in 2016 and an age-stan-
dardized death rate of 1.5 per 100,000 individuals [2]. In Taiwan, the age-adjusted incidence of MM increased by 13% from 2007 to 2012, that is, from 1.41 to 1.59 per 100,000 individuals. After the introduction of novel agents including bortezomib, thalidomide, and lenalidomide in Taiwan, there was a marked change in treatment patterns, but fatality rates in patients with MM remained high (19.4%) [3].

The cause of MM is unclear; however, exposures to organic solvents, such as benzene, radiation, chronic antigen stimulation, and genetic factors have been linked to increased risk [4]. Several genetic factors have been implicated in MM: (i) trisomies of one or more of the odd chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 are oncogenic pathways; (ii) dysregulation of the family of cyclin D protein (cyclins D1, D2, and D3) are an abnormality present at the very early stages of MM development; (iii) translocations involving the IgH locus (14q32) and multiple other genes, including cyclins D1 (CCND1 and 11q13), cyclins D3 (CCND3 and 4p16), FGFR3-MMSET (6p21), MAF (16q23), and MAFB (20q11); and (ix) deletion of the short arm of chromosome 17 causing the loss of tumor suppressor TP53 activity [1].

Gaucher disease (GD, MIM 230800), an autosomal recessive lysosomal storage disorder that is the most common glycolipid storage disorder, results from the inherited deficiency of the lysosomal enzyme acid β-glucosidase (glucocerebrosidase, GBA, E.C.3.2.1.45). Enzymatic deficiencies alter the degradation of glycosphingolipids and result in the accumulation of glucosylceramide, mainly within cells of the monocyte/macrophage lineage, causing a complex biochemical change in the organellar, cellular, and tissue levels. These changes result in progressive bone disease, cytopenias, and hepatosplenomegaly, which may present at any age [5–8].

On the basis of the age of onset, clinical signs, and involvement of neurological symptoms, GD has been subdivided into three clinical categories. Type 1 (adult type, chronic, non-neuronopathic), the most common, is characterized by enlargement of the internal organs and the lack of central nervous system involvement. Type 2 (infantile, acute neuronopathic) is rare, and is characterized by the early appearance of visceral signs, neuronopathic fulminant, and death a few months after birth. Type 3 (subacute neuronopathic) is characterized by the early onset of visceral impairment and chronic neuronopathic progression [9]. Due to the relatively long life expectancy of patients with GD type 1, their chances of developing cancer are also much higher than those of patients with type 2 or 3 GD [10].

The risk of developing gammopathies, including polyclonal gammopathy, monoclonal gammopathy of undetermined significance, and MM in patients with GD, is higher in those with MM, compared to that in the general population [5, 6, 11]. In the general population, the risk of transformation of monoclonal gammopathy of undetermined significance to MM is estimated to be 1% per year [12], but no data are available for the global GD population [13]. The risk of monoclonal gammopathy of undetermined significance and MM increases with age in both the general population and patients with GD [5, 6, 11, 14], but the overall risk of MM in GD is 5.9–51.1 times higher than that in the general population [5, 7].

This higher risk of cancer may be related to GBA mutations. When GBA activity decreases, ceramide content is greatly reduced, and glucosylceramide accumulates and promotes the synthesis of sphingosine-1-phosphate and ceramide-1-phosphate. Ceramide is a strong tumor suppressor, while sphingosine-1-phosphate and ceramide-1-phosphate promote cell proliferation and differentiation. Over the long term, the metabolic balance of ceramide/glucosylceramide/sphingolipids is disrupted, increasing the risk of cancer [15]. In this study, we hypothesized that altered metabolism of ceramide/sphingolipids could be a possible cause of MM. Therefore, we focused on a small cohort of patients with MM and assayed for variants of the GBA gene to test this hypothesis.

Materials and Methods

Study Samples

Samples were obtained from the Human Biobank, China Medical University Hospital (Taichung City, Taiwan). All experiments were conducted in compliance with government laws and ethics. The keyword “multiple myeloma” was used to search for the diagnosis in the biobank, which contained a total of 24 samples (buffy coat or DNA). Of these samples, 20 were male, 4 were female; the average age of diagnosis was 56.4 year (range: 40–73). Genomic DNA was extracted from buffy coat samples using the MagNA Pure LC DNA Isolation Kit (Roche, Mannheim, Germany) according to the manufacturer’s instruction. DNA concentration was adjusted to 10 ng/μL for PCR amplification.

PCR Amplification

Because a highly homologous 5.7-kb pseudogene (GBAP1) is located approximately 16 kb downstream of the true functional GBA gene, a two-step PCR method was performed as described previously, with some modifications [16, 17]. In the first step of PCR, all exonic sequences and most intronic sequences were selectively amplified in three fragments ranging from 1.7 kb to 3 kb in length using EmeraldAmp MAX PCR Master Mix (Takara Bio Inc. Kusatsu, Shiga, Japan). A fragment encompassing exons 2–6 was amplified using the forward primer 5′-CCTAAAGTTGTCAGCTCCTAAAGTTGTCA-
Gene were observed: IVS2+236A>C in the GBA gene, passing the two single-nucleotide polymorphisms (SNPs) encompassing the coding regions of GBA in any of the 24 study subjects. No single-nucleotide polymorphisms (SNPs) were recorded in dbSNPs (dbSNPs_East Asian population) or searched in the Human Gene Mutation Database [18], ClinVar [19], dbSNPs [20], and Taiwan Biobank [21] to determine if they have been reported before and to look for additional clinical information. Novel variants were subjected to in silico analysis using prediction tools (SIFT [22] and PolyPhen-2 [23]) to evaluate the possibility of DNA damage.

Statistical Analysis

Multiple parameters were compared using χ2 test. Statistical significance was set at p < 0.05. Statistical calculations were performed using SPSS software (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

Directed DNA sequencing revealed no variants in the coding regions of GBA in any of the 24 study subjects. Two single-nucleotide polymorphisms (SNPs) encompassing the GBA gene were observed: IVS2+236A>C (rs2070679) and IVS2+307T>C (rs2361534). These two variants were not in coding sequences and were not reported in the Human Gene Mutation Database and ClinVar database. The minor allele frequencies (MAF) of these two SNPs were 4.2% (2/48) and 2.1% (1/48) in the patient cohort. The MAF of these two SNPs recorded in public databases served as control group. Since the genotyping data generated by Taiwan Biobank were based on SNP microarray, not every SNP was designed and linked to MAF information. Since information on rs2070679 was not available in Taiwan Biobank, we use the MAF recorded in dbSNPs_East Asian population (3.67%, 37/1,008); for rs2361534, the MAF was 0.17% (5/3,030) in the Taiwan Biobank and 0.10% (1/1,008) in dbSNPs_East Asian population. Therefore, we used the MAF recorded in the Taiwan Biobank record for comparison. No significant difference was found in the MAF of rs2070679 (p = 0.8921), the C allele frequency was 2/48 (4.2%) in the patient cohort and 37/1,008 (3.67%) in the control group. No significant difference was found in the MAF of rs2361534 (p = 0.0028); the C allele frequency was 2 (4.2%) in the patient cohort and 37 (3.67%) in the control group.

Discussion

Multiple myeloma is one of the most common hematological malignancies in humans. Approximately 114,500 individuals are diagnosed with MM worldwide annually, and approximately 80,000 people die from it annually, and approximately 80,000 people die from it.
annually [3]. As observed in our patient cohort (20 males, 4 females), males are more often affected than females, and the incidence increased with age. The incidence of MM is higher in Western countries than in Asian countries; however, in the past few decades, the incidence of MM has increased significantly in the latter [3]. This increase has been attributed to factors such as population aging and population growth; environmental pollution and exposure to chemical solvents also increase the risk of MM. Moreover, genetic factors are associated with the onset of MM [24–28]. Currently MM can be treated by several methods, including chemotherapy, drugs such as thalidomide, bortezomib, and lenalidomide, and bone marrow transplantation; however, early detection and treatment and understanding of the pathogenic mechanism of MM can help improve the prognosis of MM treatment [29]. In a recent case report, a 30-year-old man diagnosed with MM presented with splenomegaly and a DNA test revealed that he was a GD carrier. They speculated that the GD carrier status could be attributed to splenomegaly and the Jewish heritage of the patient [4]. This case suggested the possibility of association between MM and GBA gene mutations.

Gaucher disease has been linked to certain types of cancers, especially myeloma [15, 30]. To date, two mechanisms have been proposed to underlie increased tumorigenesis associated with GD. In the first, a deficiency in GBA enzyme activity results in glucosylceramide accumulation in the organelles, late endosomes, and lysosomes of macrophages (known as Gaucher cells) in many organs. Chronically, alternatively activated GCs can secrete several anti-inflammatory and proinflammatory chemokines, cytokines, and hydrolases that disturb cellular and cytokinic local microenvironments. These disturbance lead to the dysregulation of immune function, facilitating carcinogenesis (Fig. 1) [15, 31]. In the second mechanism, an imbalance between pro- and antiproliferative sphingolipid arises in the cell. Ceramide, a GBA

Fig. 1. Schematic of ceramide/glucosylceramide metabolism and its biological functions in cell survival and apoptosis. Arrows indicate the direction of compound synthesis. DES, dihydroceramide desaturase; SMase, sphingomyelinase; SMS, sphingomyelin synthase; C1PP, ceramide-1-phosphate phosphatase; CK, ceramide kinase; CDase, ceramidase; CS, ceramide synthase; GBA, acid β-glucosidase; GCS, Glucosylceramide synthase; S1PP, sphingosine-1-phosphate phosphatase; SK, sphingosine kinase; S1PL, sphingosine-1-phosphate lyase

GBA Variants in Patients with Multiple Myeloma from Central Taiwan

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metabolite, is a powerful tumor suppressor, which can induce autophagy, apoptosis, and cell cycle arrest [32, 33]. When ceramide concentration decreases, cancer cells show increased proliferation. In addition, the accumulation of glucosylceramide activates another metabolic pathway of sphingolipids to produce sphingosine-1-phosphate and ceramide-1-phosphate. This pathway can contribute to cell growth, division, and survival of cancer cells (Fig. 1) [15, 30, 34–36].

Based on the results of newborn screening in Taiwan in the past few years, when GBA enzyme activity is equal or lower than 2 μmol/L/h, two pathogenic mutations in the GBA gene are used to diagnose GD; if the GBA activity is between 3 and 7 μmol/L/h, usually only one pathogenic mutation, which determines if he or she is a carrier of GD, can be identified. GBA activity equal or higher than 7 μmol/L/h is considered to be normal [37, 38]. Additionally, according to the screening data, the incidence of GD in Taiwan was approximately 1 in 300,000 individuals. Under the assumption that there was no change in the ethnic group, using the Hardy–Weinberg equilibrium model, the carrier rate of GBA in Taiwanese was calculated to be approximately 0.36%. In a recent study, Parkinson’s disease (PD) has been reported to be associated with GBA mutations, with carrier rate ranging from 3% to 8% depending on the population [34, 39]. The prevalence of MM is lower than that of PD (1–2 per 1,000 individuals in PD, 1.5–5 per 100,000 individuals in MM) [3, 40, 41] and higher than that of GD. Therefore, to identify patients MM carrying GBA mutations, the carrier rate of GBA mutation in MM should be between GD and PD carrier rate; for example, in central Taiwan, it would be 0.36–3%. In this study, the size of the MM cohort was small, and we did not observe any variants in the GBA coding region; however, the proportion could be reconsidered when more samples were available. In addition, long-time frozen blood or DNA samples were used to analyze the GBA gene, but GBA enzyme activity could not be measured from these samples. In future studies, GBA enzyme activity should be measured from fresh blood samples of patients with MM. Moreover, the correlation between MM and GBA mutations could be established by performing enzyme activity measurements and in-depth gene and function analysis, such as analyses of DNA variants, transcriptional regulation, and posttranscriptional or posttranslational modification.

Although we did not find any mutations in the coding region of GBA from the MM cohort, interestingly, the MAF of one SNP (rs2361534) located in intron 2 was higher than that of control group. Although the clinic effect of rs2361534 is not clear, this phenomenon suggests that some transcribed or translated regions around this SNP, including GBA, might be related to MM. This finding warrants further study. The other SNP, rs2070679, was also found in several GBA gene resequencing study with PD patients in which no significant association was reported [42, 43].

PCR-directed sequencing can detect only a small number of nucleotide changes in genes. In this study, although no mutation was identified in the patient cohort, it does not necessarily exclude the possibility of other variants of the GBA gene, such as inversions, translocations, and mutations within the intron and promoter. To detect these abnormalities, more elaborate sequencing would have to be performed.

In conclusion, patients with GD have a higher risk of developing cancer, especially hematological malignancy including MM. However, the relationship between MM and GBA gene mutations remains unclear. In this study, the sample size was limited and data on the enzymatic activity of GBA in patients with MM were not available. Thus, we could not establish a direct correlation between MM and GBA mutations. Therefore, a large sample size is required to perform detailed analyses and elucidate their relevance.

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Statement of Ethics

This study was approved by Research Ethics Committee, China Medical University Hospital, Taichung, Taiwan (CMUH REC No.: CMUH108-REC3-099). All research is conducted in compliance with government laws and ethics.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Fuu-Jen Tsai contributed to the conception and design of the study, interpretation of data, drafting and revising the manuscript, and approval of the version of the manuscript to be published.

Wei-De Lin contributed to the acquisition of data, analysis of data, drafting and revising the manuscript, and approval of the version of the manuscript to be published.

References

1 Brigle K, Rogers B. Pathobiology and diagnosis of multiple myeloma. Semin Oncol Nurs. 2017 Aug;33(3):225–36.
2 Cowan AJ, Allen C, Barac A, Basaleem H, Benson I, Curado MP, et al. Global burden of multiple myeloma: a systematic analysis for the global burden of disease study 2016. JAMA Oncol. 2019 Sep;5(9):1221–7.
3 Tang CH, Liu HY, Hou HA, Qiu H, Huang KC, Siggins S, et al. Epidemiology of multiple myeloma in Taiwan, a population based study. Cancer Epidemiol. 2018 Aug;55:136–41.
4 Chadha J, Sahai T, Schwimmer J, Shani D. A 30-year-old carrier of gaucher disease with multiple myeloma. Case Rep Oncol Med. 2018 Sep;15(4):5.
5 Rosenbloom BE, Weinreb NJ, Zimran A, Kacena KA, Charrow J, Ward E. Gaucher disease and cancer incidence: a study from the gaucher registry. Blood. 2005 Jun 15;105(12):4569–72.
6 de Fost M, Vom Dahl S, Weverling GJ, Brill C, Rogers B. Pathobiology and diagnosis of Gaucher disease. Br J Haematol. 2007 Sep;138(6):676–86.
7 Thomas AS, Mehta A, Hughes DA. Gaucher disease: haematological presentations and complications. Br J Haematol. 2014 May;165(4):427–40.
8 Pastores GM, Hughes DA. lysosomal storage disorders and malignancy. Diseases. 2017 Feb 27;5(1):18.
9 Futerman AH, Sussman JL, Horowitz M, Silman I, Zimran A. New directions in the treatment of Gaucher disease. Trends Pharmacol Sci. 2004 Mar;25(3):147–51.
10 Mistry PK, Taddei T, von Dahl S, Rosenbloom BE. Gaucher disease and malignancy: a model for cancer pathogenesis in an inborn error of metabolism. Crit Rev Oncog. 2013;18:235–46.
11 Hughes D, Cappellini MD, Berger M, van der Meulen A, Curaud MP, et al. Recommendations for the management of the haematological and onco-haematological aspects of Gaucher disease. Br J Haematol. 2007 Sep;138(6):676–86.
12 Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood. 2009 May 28;113(22):5412–7.
13 Weinreb NJ, Mistry PK, Rosenbloom BE, Dhodapkar MV, MGUS, lymphoplasmacytic malignancies, and Gaucher disease: the significance of the clinical association. Blood. 2018 May 31;131(22):2500–1.
14 Anderson KC. Multiple myeloma: How far have we come? Mayo Clin Proc. 2003 Jan;78(1):1–7.
15 Wątek M, Piktel E, Wollny T, Durnaś B, Fiedoruk K, Lech-Marańda E, et al. Defective sphingolipids metabolism and tumor associated macrophages as the possible links between gaucher disease and blood cancer development. Int J Mol Sci. 2019 Feb 15;20(4):843.
16 Stone DL, Tayebi N, Orviskey S, Eustiefield B, Madike V, Sidransky E. Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease. Hum Mutat. 2000;15(2):181–8.
17 Wan L, Hsu CM, Tsai CH, Lee CG, Hwu WL, Tsai FJ. Mutation analysis of Gaucher disease patients in Taiwan: high prevalence of the RecNcl and 1444P mutations. Blood Cells Mol Dis. 2006 Jan-Feb;36(1):53–8.
18 Thorpe JW, Maton JA, Caporaso NE, Hayes RB, et al. Genetic factors influencing susceptibility to multiple myeloma. Nat Commun. 2018 Sep 13;9(1):3531–30.
19 Wątek M, Piktel E, Wollny T, Durnaś B, Niemirowicz M, Suska A, Drudz-Sitek A, et al. Genetic polymorphisms in genes of class switch recombination and multiple myeloma risk and survival: an IMMeNSE study. Leuk Lymphoma. 2019 Jun;60(7):1803–11.
20 Tang CH, Hou HA, Huang KC, Qiu H, Liu Y. Treatment evolution and improved survival in multiple myeloma in Taiwan. Ann Hematol. 2020 Feb;99(2):321–30.
21 Barth BM, Shanmugavelan SSD, Taselkosky DM, Kester M, Morad SA, Cabot MC. Gaucher’s disease and cancer: a sphingolipid perspective. Crit Rev Oncog. 2013;18(3):235–46.
22 Boven LA, van Meurs M, Boot RG, Mehta A, Boon L, Aerts JM, et al. Gaucher cells demonstrate a distinct macrophage phenotype and resemble alternatively activated macrophages. Am J Clin Pathol. 2004 Sep;122(3):359–69.
23 Morad SA, Cabot MC. Ceramide-orchestrated signalling in cancer cells. Nat Rev Cancer. 2013 Jan;13(1):51–65.
24 Astudillo L, Therville N, Colacios C, Ségui B, Andrieu-Abadie N, Laveide T. Glucosylceramide-like macrophage-like and anti-macrophage like macrophages. Biochimie. 2016 Jun 15;125(67–80).
25 Stirnemann J, Belmatoug N, Camou F, Serратrice C, Frossart A, Caillaud C, et al. A review of gaucher disease pathophysiology, clinical presentation and treatments. Int J Mol Sci. 2017 Feb 17;18(2):441.
26 Wollny T, Wątek M, Durnaś B, Niemirówicz K, Piktel E, Żendzian-Piotrowska M, et al. Sphingosine-1-phosphate metabolism and its role in the development of inflammatory bowel disease. Int J Mol Sci. 2017 Mar 31;18(4):741.
36. Wątek M, Pińtka E, Barankiewicz J, Sierlecka E, Kościółek-Zgódka S, Chabowska A, et al. Decreased activity of blood acid sphingomyelinase in the course of multiple myeloma. *Int J Mol Sci*. 2019 Nov 30;20(23):6048.

37. Liao HC, Chiang CC, Niu DM, Wang CH, Kao SM, Tsai FJ, et al. Detecting multiple lysosomal storage diseases by tandem mass spectrometry: a national newborn screening program in Taiwan. *Clin Chim Acta*. 2014 Apr 20;431:80–6.

38. Chiang SC, Chen PW, Hwu WL, Lee AJ, Chen LC, Lee NC, et al. Performance of the four-plex tandem mass spectrometry lysosomal storage disease newborn screening test: the necessity of adding a 2nd tier test for Pompe disease. *Int J Neonatal Screen*. 2018 Dec 18;4(4):41.

39. Lee C-C, Tsai C-H, Wan I, Tsai Y, Lin Y-J, Wang W-F, et al. Increased incidence of Parkinsonism among Chinese with β-glucosidase mutation in central Taiwan. *BioMedicine*. 2013 Jun;3(2):92–4.

40. Kazandjian D. Multiple myeloma epidemiology and survival: a unique malignancy. *Semin Oncol*. 2016 Dec;43(6):676–81.

41. Tysnes OB, Storstein A. Epidemiology of Parkinson’s disease. *J Neural Transm*. 2017 Aug;124(8):901–5.

42. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson’s disease. *N Engl J Med*. 2009 Oct;361(17):1651–61.

43. Mitsui J, Fukuda Y, Azuma K, Tozaki H, Ishiura H, Takahashi Y, et al. Multiplexed resequencing analysis to identify rare variants in pooled DNA with barcode indexing using next-generation sequencer. *J Hum Genet*. 2010 Jul;55(7):448–55.