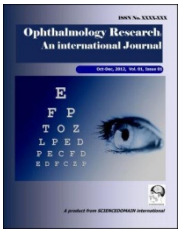

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Association of the *CASQ1* Gene SNP rs3838216 with Graves' Ophthalmopathy and Hashimoto's Thyroiditis in Patients with Thyroid Autoimmunity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JW and HL designed the study, and wrote the first draft of the manuscript. Authors HL and DC collected the data. Authors SE, LD and PC provided patients thyroid tissue specimens at thyroidectomy and blood samples for DNA extraction from patients recruited at Nepean Hospital and Royal North Shore Hospital in New South Wales. Author JPW provided DNA samples from Patients recruited at Sir Charles Gairdner Hospital in Western Australia. Author BC critically read the manuscript. Authors HL, JPW and JW managed the analyses of the study. Author HL performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the final draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The pathogenesis of Graves' ophthalmopathy is poorly understood, but there is evidence for the involvement of calsequestrin (*CASQ1*) as an autoantigen.
Aim: To compare the frequency of the single nucleotide polymorphism (SNP) rs3838216

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(located in intron 1 of *CASQ1*) in patients with autoimmune thyroid disease (ATD), Graves' Ophthalmopathy and controls.
Methods: Germline DNA was assayed for rs3838216 by MassARRAY SNP analysis using iPLEX technology of SEQUENOM in 405 individuals (98 males, 307 females) with ATD (comprising Graves' Opthalmopathy (GO, N=74), Graves' Hyperthyroidism (GH, N=131), Hashimoto's thyroiditis (HT, N=92), and controls with no personal or family history of autoimmune thyroid disorders (N=108).
Results: Genotypes for rs3838216 differed significantly across groups with minor allele frequencies as follows: GO 17%, GH 24%, HT 19% and controls 30% groups (*P*=0.0427). *P* of SNP rs3838216 was significant in GO vs. control (odds ratio 2.16, *P*=0.003), and HT vs control (odds ratio 1.87, *P*= 0.008). On pair wise analysis, homozygosity for the major allele was associated with GO vs. control (odds ratio = 2.42, *P*=0.0046), and HT vs control (odds ratio 2.07, *P*=0.0116); whereas heterozygosity was associated with GO vs. control (odds ratio = 0.52, *P*=0.039), and HT vs control (odds ratio 0.570, *P*=0.054).
Conclusion: The *CASQ1* gene SNP rs3838216 is associated with autoimmune thyroid disease and with GO in particular.

Keywords: Graves' disease; Hashimoto's thyroiditis; ophthalmopathy, single nucleotide polymorphism, homozygosity; heterozygosity; CASQ1; calsequestrin 1.

ABBREVIATIONS

GO: Graves' Ophthalmopathy, GH: Graves' Hyperthyroidism, HT: Hashimoto's Thyroidities, TRAB: Thyroid Receptors Antibodies, TPO: Thyroid Peroxides. TgAB: Thyroglobulin Antibodies, TSH: Thyroid Stimulating Hormone, EUGOGO: European Group on Graves' Orbitopathy.

1. INTRODUCTION

The eye disorder or ophthalmopathy associated with Graves' hyperthyroidism greatly reduces the quality of life in affected patients [1,2] and can lead to eye muscle damage and loss of sight. Mild eye signs are also present in about 25% of patients with Hashimoto's thyroiditis [3,4] mainly manifesting as upper eyelid disease (retraction), and in also a small proportion of patients with transient (subacute and silent) thyroiditis [5]. Graves' disease is a multi-factorial disorder that occurs in subjects with a genetic susceptibility and may be precipitated following an external (environmental) stimulus such as infection, stress, pregnancy or trauma [6,7]. The pathogenesis of the Graves ophthalmopathy (GO) [8] and the mechanism for its link to Graves' hyperthyroidism, and the more common Hashimoto's thyroiditis is poorly understood. Our current research is focused on identifying a genetic marker for this unique association.

There are three main sub types of GO namely, congestive ophthalmopathy, ocular myopathy and mixed congestive and myopathic ophthalmopathy [4]. Congestive ophthalmopathy is characterized by inflammation of the ocular connective tissues (OCT), with no involvement of the extra ocular muscles. It is associated with increased and abnormal adipogenesis and glycosaminoglycans (GAGS) over-production in the orbital connective tissue [9,10] and manifests with clinical features of eye swelling, conjunctival injection, chemosis, watery or gritty eyes and exophthalmos. In contrast, ocular myopathy is characterized by inflammation and swelling of the extra ocular muscles and manifests as eye muscle dysfunction and

diplopia and occasionally, painful eye movements [11,12]. Although congestive and myopathic features can occur in isolation, mixed congestive and myopathic ophthalmopathy is the most common presentation of GO, occurring in approximately 60% of GO patients [4]. In patients with Hashimoto's thyroiditis, UER (Upper Eyelid retraction) and lag are often the only features of an ophthalmopathy except for mild proptosis [3]. In 10% of patients, ophthalmopathy occurs in the apparent absence of thyroid autoimmunity and TSH – receptor (TSH-R) antibodies - so-called "euthyroid Graves' disease".

The mechanism for the eye muscle component of GO has been the subject of our studies over many years [13]. The main candidate antigen is the calcium binding protein calsequestrin (CASQ1). We have shown that antibodies against CASQ1 are closely associated with eye muscle damage in patients with GO and appear to be markers for Nunery type 2 ophthalmopathy (i.e. with eye muscle involvement) [14–18]. CASQ1 antibodies are also frequently found in patients with the inflammation of the levator palpebrae superioris muscle that is commonly associated with Hashimoto's thyroiditis [19].

CASQ1 is a 367 amino acids glycoprotein with a molecular weight of ~ 67,000 on SDS-PAGE. It is found in cardiac, skeletal and smooth muscle and in other tissues and species including the cerebellum and in plant cells. It stores calcium ions in sufficient quantities to allow repetitive contractions and is essential to maintain movement, respiration and cardiac function. When calcium binds to CASQ1 there is a structural change whereby the alpha-helical content of the protein increases from 3 to 11% [20]. The human skeletal muscle *CASQ* gene, *CASQ1*, has 11 exons, located on band 1q21, and the related cardiac *CASQ* gene, *CASQ2*, located on bands 1p11-p13.3, and shares 64% amino acid homology. It was reported by Porter et al. [21] that CASQ1 was expressed in the extraocular muscle 4.7 times more than in other skeletal muscle, while others [22] showed that CASQ2 was expressed 2.7 times more. We have shown recently that antibodies against CASQ1 and CASQ2 do not share epitopes [23].

The complexity of the human genome is characterized by many types of mutations and polymorphisms, including insertion and deletions, the expansion of tandem repeat sequences and single nucleotide polymorphisms (SNP). Recent studies suggested that noncoding SNPs in *CASQ1* gene alter diabetes susceptibility [24], either by a direct effect on *CASQ1* gene expression or by regulating a nearby gene. A missense mutation, producing a single amino acid substitution in the *CASQ2* gene, suggested that *CASQ2* played a critical role in maintaining cardiac muscle function [25]. In a recent microarray study of RNAs from Graves' thyroid tissues, we identified 296 genes that were differentially expressed between Graves' hyperthyroidism with and without ophthalmopathy [26]. Of these, the cardiac calsequestrin gene *CASQ2* was the most highly expressed gene in GO. Recent results show that the CASQ1 protein is expressed in thyroid tissue, (Cultrone, Lahooti, Wall et.al., 2014 In Press) raising the possibility that skeletal muscle *CASQ1* polymorphisms expressed in thyroid tissues may be associated with skeletal muscle myopathies including the extra ocular muscles components of GO.

2. MATERIALS AND METHODS

2.1 Clinical Subjects

A comprehensive demographic, clinical details and genotypes of SNP rs3838216 for patients with thyroid autoimmunity and control subjects without autoimmune diseases (studied) are

summarized in Table 1. The median age in years for 98 male patients (24 %) was 37 years with range between 17 to 92 years old and for 307 female patients (76 %) the median age year was 50.5 years with range between 16 to 92 years old. Patients were with Graves' hyperthyroidism with ophthalmopathy (GO), Graves' hyperthyroidism without ophthalmoapthy (GH) or Hashimoto's disease (HT). Control group were subjects with no known history of autoimmune thyroid disorders. Participants of this cohort were recruited from outpatients' clinic at Nepean and Royal North Shore Hospitals in New South Wales and Sir Charles Gairdner hospital in Western Australia. Previous treatments for hyperthyroidism (with particular reference to radioactive Iodine therapy), gender distribution of Graves' patients with or without GO, presence or absence of other autoimmune diseases, presence of or absence of ethnic differences in the different groups of patients are shown in Table 1. Patients' recruitment criteria are described by Walsh et. al., 2011. Patients in Perth cohort were assessed by John Walsh and patients in Sydney cohort were assessed by Jack Wall, both endocrinologist have particular interest in thyroid eye disease, and clinical severity of disease was assessed according to EUGOGO criteria where mild =1, moderate – severe = 2 and sight – threatening = 3 (Bartalena et.al., 2008). Smoking data was not specifically collected in the study. Ethical approval was obtained from Nepean Blue Mountain Local Health District and Sir Charles Gairdner Hospital Human Research Ethic Committees.

2.2 Methods

Five hundred ng of genomic DNA extracted from leucocytes of 405 patients and normal subjects, dried in a 96 well microtitre plate for MassARRAY SNP analysis using iPLEX technology of SEQUENOM, tested for by Australian Genome Research facility (AGRF). All reactions for the iPLEX assay are terminated after a single base extension, this single base primer extension reactions coupled with MALDI-TOF MS for multiplexed genotyping, while simplify genotyping. DNA was prepared from blood lymphocytes in this way and analyzed for *CASQ1* SNPs.

The *CASQ1* genome composes of 11 exons, and 10 introns. Search of Bioinformatics SNP database (http://www.ncbi.nlm.nih.gov/snp/) showed that there were 246 SNPs in the human calsequestrin gene (*CASQ1*). Alignment of *CASQ1* genes of several species such as lampreys, fugue, xenopus, dog, marsupial mouse, and rhesus monkey, with human *CASQ1* gene, identified conserved elements containing highly conserved polymorphisms (http://ecrbrowser.dcode.org/).This conservation points to evolutionary importance and possible pathogenicity of these SNP polymorphisms. The alignment of the *CASQ1* genes showed 33 single nucleotide polymorphisms that appeared to be highly conserved. Four of these (rs7412379, rs3747623, rs3838216, and rs2275703) potentially pathogenic SNPs, or informative SNPs, were identified in DNA samples from 405 patients and subjects with no known history of autoimmune thyroid disorders.

SNP rs3838216 [-ggcatt...] is not a mere single nucleotide polymorphisms, rather it is an insertion or deletion of 6 nucleotide occurring in intron 1, which potentially could affect the promoter region of *CASQ1* gene in trans and subsequently affecting the *CASQ1* gene transcription. Therefore, with respect to its genotypes and allelic frequencies, was further studied here. In wild type Genotype the sequence [-ggcatt...] is absent in both strands of DNA (absent–absent or -/-), heterozygote genotype has sequence [-ggcatt...] absent in one strand while this sequence is present in the parallel strand (-/-ggcatt.. or -/+.). In the homozygote, sequence [-ggcatt...] is present in both strands of DNA (-ggcatt.../-ggcatt... or +/+).

Table 1. Demographics, clinical details, of patients with thyroid autoimmunity and control subject without autoimmune disease

Control	Graves Ophthalmopathy	Graves Hyperthyroidism	Hashimoto's Thyroiditis
***Age: 35.7±12.8 (**N=108 26%)**	***Age: 52.4±14.6 (**N=74 18%)**	***Age: 48.7±15.8 (**N=131 32%)**	***Age: 51.8±15.3 (**N = 92 23%)**
Clinical Parameter			
Age at Diagnosis	43 ± 14 (N = 66)	40±16 (N=116)	39±17 (N=99)
Other Autoimmune disorders	7 (41%)	11 (7%)	17 (15%)
Family History of Thyroid Disorders	24 (66%)	77 (54%)	57 (56%)
Isotope Scan	21 (40%)	52 (71%)	
Goiter diffuse with 1 -3 nodules	23 (38%)	29 (47%)	
1 Nodule	10 (29%)	19 (27%)	49 (47%)
2 Nodules	15 (44%)	39 (56%)	55 (53%)
Medication	51 (60%)	88 (61%)	107 (92%)
Radioactive iodine treatment.	12 (14%)	21 (14%)	
Surgery	22 (26%)	36 (25%)	
TRAB	46.4±132 (N=30)	65.5±160 (N=37)	
TPO	136±106 (N=6)	752±876 (N=10)	904±899 (N=62)
FT4 at Diagnosis	37.7±23.7 (N=40)	45.2±27.5 (N=74)	12.2±6.1 (N=47)
FT3 at Diagnosis	17.1±11.9 (N=32)	35.8±124 (N=51)	17.3±33.2 (N=6)
TSH			26.9±39.8 (N=86)
TgAB			169±286 (N=35)
Non-European	N=7	N=32	N = 7
Male	N=12	N=30	N = 12
Female	N=62	N=101	N = 84
Severity: EUGOGO	1–3 (N=58)		

** Age of population expressed as mean years ± SD; ** N = Number of patients expressed as % of total population; TRAB: Thyroid Receptors Antibodies, TPO: Thyroid Peroxides. TgAB: Thyroglobulin Antibodies, TSH: Thyroid Stimulating Hormone, EUGOGO: European Group on Graves' Orbitopathy. FT4: FREE T4, FT3: FREET3*

2.3 Statistical Analyses

Genotype and allelic frequencies of the *CASQ1* SNP rs3838216 in patients with Graves' hyperthyroididsm, Graves ophthalmopathy, Hashimotos thyroiditis and control subjects, were compared using X^2 test or Exact Fisher's test following Hardy – Weinberg equilibrium with statistical package Graph Pad version 3. A two-tailed *P*-value less than 0.05 were considered significant.

4. RESULTS AND DISCUSSION

4.1 Results

Analysis of SNP rs3838216 across the three genotypes for GO, GH, HT and control groups (Table 2) showed significant probability (*P = 0.0427). Presence of sequence [-ggcatt...] in one of the DNA strands in heterozygote genotypes and in both strands of DNA of homozygote genotypes represent minor allele and the frequency for GO, GH, HT and control were 17%, 24%, 19% and 30% respectively.

Allele frequencies for SNP rs3838216 in patients and control groups shown in Table 3 were in Hardy-Weinberg equilibrium. Significance of the differences in distribution of allelic frequencies for SNP rs3838216 were; for GO vs. GH [odds ratio 1.59, 95% confidence interval (0.95-2.66), *P*=0.075], GO vs. control [odds ratio 2.16, 95% confidence interval (1.29-3.64), ** P = 0.003] and HT vs. control [odds ratio 1.87, 95% confidence interval (1.17–2.99), **P=0.008]. No significant difference in alleles distributions frequencies were found for; GH vs. control (odds ratio 1.36, 95% confidence interval (0.91-2.04), *P*=0.134], and GH vs. Hashimoto's thyroiditis [odds ratio 0.727, 95% confidence interval (0.457-1.16), P = 0.176].

We were interested to know if individual genotypes would show significant probability and risk ratio in GO, GH, HT and control groups. Therefore, we performed pair wise analysis to determine the probability and odds ratio for each genotype separately shown in Table 4. The difference in genotype frequencies for the wild type observed in GO vs. control was significant [odds ratio 2.42, 95% confidence interval (1.30-4.48), **P=0.004] and HT vs. control [odds ratio 2.07, 95% confidence interval (1.17–3.67), **P=0.011]. While that for GH vs. control subjects [odds ratio, 1.55, 95% confidence interval (0.93–2.59), *P*=0.091] failed to reach statistical significance.

A significant difference in frequency for heterozygote observed for GO vs. control [odds ratio 0.523, 95% confidence interval (0.29–1.00), *P=0.039] and HT vs. control [odds ratio 0.608, 95% confidence interval (0.35–1.10), *P=0.054,] Table 5.

A difference in frequency for homozygote observed for GO vs. control [odds ratio 0.167, 95% confidence interval (0.021–1.40), *P*=0.085] which failed to reach statistical significance Table 6.

Table 2. Genotype of *CASQ1* rs3838216 SNP distribution in patients with autoimmune thyroid disease with and without Ophthalmopathy and controls

Patients group	Wild type		Heterozygote		Homozygote		Total		MAF	X^2	*P*-value
	*Number	%	*Number	%	*Number	%	*Number	%			
GO	50	(68)	23	(31)	1	(1)	74	(100)	17%		
GH	75	(57)	48	(37)	8	(6)	131	(100)	24%		
HT	59	(64)	31	(34)	2	(2)	92	(100)	19%		
Control	50	(46)	50	(46)	8	(8)	108	(100)	30%		
Total	239	(58)	151	(37)	19	(5)	405	(100)		13.02	0.042

*GO = Graves' Ophthalmopathy; GH = Graves' hyperthyroidism; HT = Hashimoto's thyroiditis; MAF = Minor Allele Frequency; * N = Number of patients expressed as % of each population*

Table 3. Major and Minor alleles distribution of *CASQ1* rs3838216 SNP in patients with autoimmune thyroid disease with and without ophthalmopathy and controls

Patients group	Number of major alleles	Number of minor alleles	X^2	Odds ratio (95% confidence interval)	*P*-value
GO	123	25	3.16	1.59	0.075
GH	198	64		(0.95-2.66)	
GO	123	25	8.74	2.16	0.003
Control	150	66		(1.29-3.6)	
GH	198	64	2.24	1.36	0.1340
Control	150	66		(0.91-2.04)	
HT	149	35	7.003	1.87	0.008
Control	150	66		(1.17–2.99)	
GH	198	64	1.83	0.73	0.176
HT	149	35		(0.46-1.16)	

GO = Graves' Ophthalmopathy; GH = Graves' hyperthyroidism; HT = Hashimoto's thyroiditis

Table 4. Wild type Genotype of *CASQ1* rs3838216 SNP distribution in patients with autoimmune thyroid disease with and without Ophthalmopathy and controls

Patients Group	Wild type		Variant		X^2	Odds ratio (95% confidence interval)	*P*-value
	*Number	(%)	*Number	(%)			
GO	50	(24)	24	(12)	2.11	1.55	0.146
GH	75	(36)	56	(28)		(0.85-2.80)	
GO	50	(28)	24	(13)	8.03	2.42	0.004
Control	50	(28)	58	(31)		(1.30-4.48)	
GH	75	(32)	56	(23)	2.85	1.55	0.091
Control	50	(21)	58	(24)		(0.93-2.59)	
HT	59	(29)	33	(17)	6.37	2.07	0.011
Control	50	(25)	58	(29)		(1.17–3.67)	
GH	75	(34)	56	(25)	1.06	0.75	0.302
HT	59	(26)	33	(15)		(0.43-1.30)	

*1. GO = Graves' Ophthalmopathy; 2. GH = Graves' hyperthyroidism; 3. HT = Hashimoto's thyroiditis; P = < 0.1 indicates a tendency towards the significance; * N = Number of patients expressed as % of total population of each pair*

Table 5. Heterozygous Genotype of *CASQ1* rs3838216 SNP distribution in patients with autoimmune thyroid with and without Ophthalmopathy and controls

Patients group	Heterozygote		Without Heterozygote		X^2	Odds ratio (95% confidence interval)	*P*-value
	*Number	(%)	*Number	(%)			
GO	23	(13)	51	(28)	4.23	0.523 (0.29-1.00)	0.039
Control	50	(27)	58	(32)			
GO	23	(11)	51	(25)	0.646	0.780 (0.43-1.43)	0.422
GH	48	(24)	83	(40)			
GH	48	(20)	83	(35)	2.28	0.671 (0.40-1.13)	0.131
Control	50	(21)	58	(24)			
HT	31	(15)	63	(31)	3.71	0.608 (0.35-1.10)	0.054
Control	50	(25)	58	(29)			

*1. GO = Graves' Ophthalmopathy; 2. GH = Graves' hyperthyroidis; 3. HT = Hashimoto's thyroiditis; P = < 0.1 indicates a tendency towards the significance; * N = Number of patients expressed as % of total population of each pair*

Table 6. Homozygous Genotype of *CASQ1* rs3838216 SNP polymorphism distribution in patients with autoimmune thyroid disease with and without Ophthalmopathy and controls

Patients group	Homozygote		Without Homozygous		X^2	Odds ratio (95% confidence interval)	P-value
	*Number (%)		*Number	(%)			
GO	1	(1.0)	73	(40)	Fisher's	0.167	0.085
Control	8	(4.0)	100	(55)	Exact test	(0.021-1.40)	
GO	1	(1.0)	73	(35)	Fisher's	0.208	0.161
GH	8	(4.0)	123	(60)	Exact test	(0.026-1.72)	
GH	8	(3.0)	123	(52)	0.160	0.813	0.690
Control	8	(3.0)	100	(42)		(0.30-2.24)	
HT	2	(1.0)	90	(45)	Fisher's	0.278	0.112
Control	8	(4.0)	100	(50)	Exact test	(0.057–1.34)	

*1. GO = Graves' Ophthalmopathy; 2. GH = Graves' hyperthyroidism; 3. HT = Hashimoto's thyroiditis; P=< 0.1 indicates a tendency towards the significance; * N = Number of patients expressed as % of total population of each pair*

5. DISCUSSION

The role(s) genetic factors plays in development of GO remains unclear. Recent work has focused on identifying genetic changes associated with GO, through small-scale case-controlled association studies with candidate genes. Several susceptibility loci/genes have been identified that are unique to GD and HT. There are genes that are common to both autoimmune thyroid diseases, indicating that there is shared genetic susceptibility to both GD and HT. The results of association studies published so far are inconclusive as the vast majority have been underpowered and poorly characterized [27-28].

To prove that this polymorphism is significantly associated with thyroid disorders with and without ophthalmopathy, we measured the rs3838216 across the three genotypes of wild type, heterozygote and homozygote for GO, GH, HT and control groups which showed a significant probability shown in Table 2, *P*=0.042. Having established the significance of this polymorphism in thyroid autoimmune disorders including ophthalmopathy, our next question was whether this polymorphism is significantly associated with ophthalmopathy. To answer this question we performed two types of statistical analysis, one we looked for alleles frequencies distribution of rs3838216 polymorphism in pair wise assessment of GO v control, GH v control, HT v Control and GH v HT, results shown in Table 3. Significant distribution of alleles frequencies for SNP rs3838216 observed in GO v control with *P*=0.003 and odds ratio of 2.16 with 95% confidence interval of 1.29-3.64. Significant allele frequencies distribution were also observed for HT v control with *P*=0.008, odds ratio of 1.87 with 95% confidence interval of 1.17-2.99. No significant difference in alleles distribution frequencies were found for GH v control or GH v HT.

Next to confirm this significant probability and odds ratio observation in GO and HT, we performed pair wise analysis of individual genotype and assessed the probability and odds ratio in wild type genotype. It can be seen in Table 4 in GO v control the probability was significant *P*=0.004 with odds ratio 2.42 with confidence interval of 1.30–4.48. Such high probability and odds ratio have been reported for HLA gene. Similarly wild type genotype showed significant probability *P*=0.011 and odds ratio of 2.07 with confidence interval of 1.17-3.67 for HT v control.

Above results for wild type genotype of rs3838216 was replicated for heterozygote genotype and results are shown in Table 5. Our data clearly shows significance of SNP rs3838216 in thyroid autoimmune disorders and in particular in Graves' ophthalmopathy, therefore, we suggested involvement of this polymorphism in thyroid eye disease. It should be noted that Graves ophthalmopathy is a complex disorder and several genes including HLA gene reported to act as risk factor for this disease. Our *CASQ1* gene SNP rs3838216 is one of the genes that we believe is a risk factor for thyroid eye disease.

Here, we showed SNP rs3838216 in the *CASQ1* gene is associated with HT and GO. At the genotypic level, results show significant probabilities and risk ratios for an association of SNP rs3838216 to GO, and HT. Our data shows risk ratios as comparable to other genes postulated to be involved in Graves' disease [29], and equates to those for HLA, thyroid stimulating receptor and thyroglobulin genes risk ratios [29]. To characterize patients in this cohort having autoimmune thyroid diseases, we included data for various established risk factors such as ethnicity, family history of thyroid disorders, gender differences, other immune disorders, FT4, FT3, TRAB, TPO, TgAB, drugs treatments, radiation therapy and surgical interventions [30]. The limitation of this study is lack of patients smoking history

which unfortunately was not recorded and it is a well known risk factor for development of GO and GH [31].

This is the first report showing association of *CASQ1* a skeletal muscle orbital autoantigen gene polymorphism in Hashimoto's thyroiditis and Graves' ophthalmopathy. In our cohort, we studied only 4 patients with Hashimoto's thyroiditis that had ophthalmopathy, but we know that 25% will have mild eye signs and UER [3], therefore, the prevalence of the SNP in patients with Hashimoto's thyroiditis is probably underestimated.

No human disease has yet been associated with mutations in the protein coding sequence of the *CASQ1* gene. It is possible that SNPs in the skeletal muscle *CASQ1* will be associated with skeletal muscle myopathies. The human skeletal muscle *CASQ1* gene has 11 exons, located to chromosome 1q2. Linkage disequilibrium studies have shown that this region of chromosome 1 and SNPs rs617599 and rs617598 variants within intron 2 are associated with type 2 diabetes [24,32]. This report suggested that noncoding SNPs in *CASQ1* alter diabetes susceptibility, either by a direct effect on *CASQ1* gene expression or by regulating a nearby gene. *CASQ1* knockout mice showed that *CASQ1* is a candidate gene for malignant hyperthermia and exertion/environmental heat stroke. Exposure of *CASQ1* null mice to either anaesthetics or heat stress resulted in elevated core body temperature, whole body contractures, and severe rhabdomyolysis [33–35]. Patients' recruitment criteria are described by Walsh et. al., 2011 [36], clinical severity of disease was assessed according to EUGOGO criteria where mild =1, moderate – severe = 2 and sight – threatening = 3 (Bartalena et. al., 2008) [37].

The functional consequence of the variant SNP rs3838216 within intron 1 of the *CASQ1* gene reported here has been studied with regard to proteins levels of CASQ1 in thyroid tissue (manuscript in press), and this variant may affect mRNA splicing or expression. We postulated that antibodies against TSH-R cause fibroblast inflammation, which leads to muscle fibre damage, release of CASQ1 protein and progression of autoimmunity in those patients with thyroid disorders who are genetically predisposed. GO is a complex polygenetic disease with many environmental triggers and risk factors. SNPs rs3838216 appears to be one of these factors. Replication of our results in a separate population of patients (racial and ethnic groups) and controls is required to confirm our findings.

6. CONCLUSION

Based on its evolutionary conservation, we postulate that *CASQ1* gene SNP rs3838216 is a genetic marker for Graves' ophthalmopathy and Hashimoto's thyroiditis autoimmune thyroid disorders. It is potentially informative genetic marker for the eye muscle component of GO. Larger populations of patients and controls of different ethnic backgrounds need to be studied to confirm detection of wild type, heterozygote and homozygote SNP rs3838216 in the *CASQ1* gene for Graves' ophthalmopathy and thyroid autoimmune disorders.

CONSENT

Local Ethical Committee approval was received for the study and informed consent of participating subjects was obtained.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Nepean Blue Mountains Local Health District scientific and ethics committees and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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