The rs2167270 polymorphism of leptin gene is associated with atopic dermatitis

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ABSTRACT
Atopic dermatitis is a chronic inflammatory skin disease that arises because of complex environmental, immunological, and genetic interactions. Adipokines are bioactive mediators secreted from adipocytes of white adipose tissue and are known to have a role in body metabolism and regulation of immune responses. Leptin is a proinflammatory adipokine that functions mainly to regulate food intake and energy expenditure. Few studies have implicated adipokines in the pathogenesis of atopic dermatitis. In this study, we investigated the association of three leptin gene polymorphisms: −2548G>A (rs7799039), −188 C/A (rs791620), and A19G (rs2167270), with the incidence of atopic dermatitis. One hundred and sixty-four patients and one hundred and sixty-seven age- and gender-matched controls were genotyped using the polymerase chain reaction-restriction fragment length polymorphism procedure. A significant association was found between rs2167270 and the incidence of atopic dermatitis (P < 0.05). The GG allele was more prevalent in the patients’ group with genotype frequency of 38.7%, compared to 26.1% for the control group. No significant differences were found in the genotype distribution or allelic frequency of the other two examined polymorphisms, rs7799039 and rs791620, between atopic dermatitis patients and controls (P > 0.05). The results suggest that rs2167270 might play a role in the pathogenesis of atopic dermatitis.

Introduction
Atopic Dermatitis (AD), also called eczema, is a chronic inflammatory skin disease that caused by complex environmental, immunological, and genetic interactions.1 It is characterized by dry skin, xerosis, itching, and relapsing dermatitis.2 Children are more susceptible to developing the disease with a worldwide incidence rate of 25% compared to 1–3% for adults.3 Adipokines are bioactive mediators secreted from adipocytes of white adipose tissue. They are considered signaling molecules that communicate with multiple organs such as muscle, brain, heart, and immune cells, in addition to adipose cells themselves.4,5 These adipokines include hormones (leptin and adiponectin), cytokines (tumor necrosis factor-α, interleukin-6, interleukin-10, and visfatin), and other proteins (apelin and resistin). They play major roles in numerous physiological and pathological processes, including body metabolism and immune response regulation.6 The dysregulation of adipokines secretion or production is considered a cause of multiple diseases such as obesity, type 2 diabetes, cardiovascular diseases,4,7 and other inflammatory disorders including asthma, rhinoconjunctivitis, and eczema.8 Leptin is an adipokine that functions mainly to maintain food intake and energy expenditure balance through hypothalamic regulation.9 Leptin is considered a proinflammatory cytokine and a member of the cytokine class 1 superfamily, due to structural similarity of leptin and its receptor with the class I cytokines superfamily, and their receptors.10 Leptin has a role in modulating the immune system; both innate and acquired immunity.11 Higher leptin levels have been observed during states of infection and inflammation, which may reflect its role in host defense and inflammation.12 In addition, a study showed that increased leptin concentrations were correlated with increased concentrations of inflammatory markers.13 Deficiencies in leptin...
adversely affect regulation of cytokines production, increase host susceptibility to infectious and inflammatory stimuli, and lead to a hematopoiesis defect. Leptin also maintains T-cell balance, stimulates T-cell activation, and triggers T-cell differentiation towards T-helper1 (Th1) over T-helper2 (Th2).

Allelic variabilities of the leptin gene located in ch7q31 are known to affect leptin transcription, expression, and secretion. Many single nucleotide polymorphisms (SNPs) have been recognized to affect the expression of leptin. For example, a common promoter polymorphic site rs7799039 SNP has been associated with transcriptional activity, a study found that AA homozygous carriers had higher levels of serum leptin than GA/GG carriers, and mRNA levels in AA carriers were 60% higher than in GA/GG carriers. Rs2167270 is another SNP located in the 5’ untranslated region. A study of an obese population concluded that homozygous carriers of the G allele had lower leptin levels compared to carrier heterozygous or homozygous for the A-allele.

According to previous studies that linked AD to leptin serum levels and studies that linked leptin serum level with allelic variability of the leptin gene, we hypothesize an association between AD and leptin gene polymorphisms. The aim of this study was to investigate the association of three single nucleotide polymorphisms of the leptin gene (rs791620, rs7799039, and rs2167270) with the incidence of AD.

**Methodology**

**Subjects**

One hundred and sixty-four blood samples of AD patients and one hundred and sixty-seven age and gender-matched controls have been recruited for this study. Subjects with any skin disease or inflammatory disease rather than AD as well as obese subjects were excluded. The age range was from less than one year to 60 years.

Informed consents were obtained from all participants/guardians according to the requirements of the Institutional Review Board of Jordan University of Science and Technology. Samples were collected from King Abdullah University Hospital, Health center at Jordan University of Science and Technology, and Ministry of Health Hospitals in North of Jordan, in the period between September 2013 until August 2015.

**Sample collection and handling**

Five milliliters of intravenous blood samples were collected in EDTA tubes and stored at −20 C for molecular analysis purposes.

**Genetic analysis**

**DNA extraction**

DNA was extracted from peripheral blood lymphocytes using a Wizard® Genomic DNA Purification Kit (Cat# A1125, Madison, USA). The extraction process was done according to manufacturer’s instructions.

**Molecular analysis**

Three SNPs have been analyzed; LEP −2548G>A (rs7799039), −188 C/A (rs791620) and A19G (rs2167270) using polymerase chain reaction (PCR) followed by restriction enzyme length polymorphism (RFLP) technique.

PCR was used for target sequence amplification. The PCR mixture was composed of 12.5 μL of a commercial master mix (Promega, Madison, USA), 6.5 μL of Nuclease free water, 1 μL each of forward and reverse primers (5 μM), and 4 μL of genomic DNA. PCR conditions for the three SNPs are described Table 1.

The PCR products were detected by loading 5 μL of the PCR products into 2% agarose gel and running at 140 volts for 60 min

| SNP ID | Primer sequence (5’–3’) | PCR annealing T (°C) | Restriction enzyme, incubation conditions | Fragment length (bp) |
|--------|-------------------------|----------------------|------------------------------------------|---------------------|
| rs7799039 | F: TTTCCTGTAATTTTCCCCG TGA G  
      R: AAAGCAAAACAGCCCCATAAAAA | 48°C | HhaI, 37°C | AA→ 242  
      GG→ 181,61  
      AG→ 242,181,61  
      AA→ 243  
      CC→ 163,80  
      AC→ 243,163,80  
      AA→ 258  
      GG→ 186,62  
      AG→ 258,186,62 |
| rs791620 | F: GGGACATCAAGGATCTCG  
      R: CTTATGGCAGCGATTACCG  | 61°C | AscI, 37°C | AA→ 242  
      GG→ 181,61  
      AG→ 242,181,61  
      AA→ 243  
      CC→ 163,80  
      AC→ 243,163,80  
      AA→ 258  
      GG→ 186,62  
      AG→ 258,186,62 |
| rs2167270 | F: GCCCCCGGAGGTCACACTG  
      R: GGCCCGTGTGGGCTGCAAG | 62°C | MspI, 37°C | AA→ 242  
      GG→ 181,61  
      AG→ 242,181,61  
      AA→ 243  
      CC→ 163,80  
      AC→ 243,163,80  
      AA→ 258  
      GG→ 186,62  
      AG→ 258,186,62 |
Restriction enzymes were used to detect fragments’ variations by the RFLP technique in which 0.5 μL of restriction enzymes purchased from New England Biolabs (UK), 2 μL of Cut Smart buffer, as well as 2 μL of nuclease free water were incubated with 10 μL of PCR product for 4 hours at 37 C, the optimum temperature for the restriction enzymes was used. Finally, the restricted products were loaded into 2.5% agarose gels, electrophoresed at 140 volts for 80 minutes, and then visualized under UV light.

Statistical analysis

The statistical package for social studies (SPSS) version 17.0 (Chicago, IL) was used for data analysis. Using Chi-square test the genotype and allele frequencies were evaluated as appropriate. Differences were considered significant when \( P < 0.05 \).

Results

Subjects

General characteristics of participants are described in Table 2. No significant differences were found in the demographics between controls and patients.

Effect of leptin gene polymorphism on atopic dermatitis

Effect of rs791620 on atopic dermatitis

All participants were genotyped for rs791620. The wild type AA genotype was detected in 23.5% of controls versus 29.3% of patients, while the homozygous GG was detected in 18.5% of controls and 22.8% of patients. The AG genotype was the most prevalent genotype. The Chi-square analysis does not show a significant association between rs791620 and AD.

Table 3. Genotypes and alleles frequency of rs791620 in AD subjects and controls.

| Polymorphisms | Control N (%) | Patient N (%) | P-value |
|---------------|---------------|---------------|---------|
| Genotype      | Control N (%) | Patient N (%) | P-value |
| AA            | 1 (0.6%)      | 0 (0.0%)      | 0.456   |
| GG            | 7 (4.3%)      | 10 (6.3%)     |         |
| Total         | 162           | 160           |         |
| Alleles       |               |               |         |
| A             | 8 (4.7%)      | 10 (5.88%)    | 0.637   |
| C             | 161 (95.3%)   | 160 (94.2%)   |         |

Effect of rs7799039 on atopic dermatitis

All the participants were genotyped for rs7799039. The wild type AA genotype was detected in 23.5% of controls versus 29.3% of patients, while the homozygous GG was detected in 18.5% of controls and 22.8% of patients. The AG genotype was the most prevalent genotype. The Chi-square analysis does not show a significant association between rs7799039 and AD.

Table 4. Genotypes and alleles frequency of rs7799039 in AD subjects and controls.

| Polymorphisms | Control N (%) | Patient N (%) | P-value |
|---------------|---------------|---------------|---------|
| Genotype      | Control N (%) | Patient N (%) | P-value |
| AA            | 38 (23.5%)    | 49 (29.3%)    | 0.184   |
| AG            | 94 (58.0%)    | 80 (47.9%)    |         |
| Total         | 162           | 167           |         |
| Alleles       |               |               |         |
| A             | 132 (48.4%)   | 129 (48.3%)   | 0.457   |
| G             | 124 (51.6%)   | 138 (51.7%)   |         |

Effect of rs2167270 on atopic dermatitis

All participants were genotyped for rs2167270. The GG genotype was more prevalent among patients. In contrast, the AG genotype was more prevalent among the control group. A significant association was observed between rs2167270 and AD.

Discussion

Genetic variations of the leptin gene are known to affect leptin expression on both mRNA and protein levels, and certain genotypes are known to increase susceptibility to certain diseases. In this study, the possible associations of three polymorphisms in the LEP
gene (rs791620, rs7799039, and rs2167270) with AD were examined.

Results showed that rs791620 did not demonstrate any significant differences in distribution between the patient and the control groups. In addition, rs7799039 had no significant association with AD, which is consistent with a study done in 2012 that failed to find an association between rs7799039 and leptin gene expression in both the mRNA and protein serum levels.17 This SNP was also negatively associated with other inflammatory disease such as psoriasis18 and Behcet’s disease.19 However, multiple studies demonstrated a significant association between rs7799039 and leptin levels among different populations. A study done in 2000 proved that the G allele was associated with being overweight and with low leptin levels in men when compared to other alleles,20 whereas a study among Brazilian women demonstrated that the G allele was associated with high leptin levels and higher risk of obesity.21 Another study showed that AA allele carriers had significantly lower leptin levels compared to other genotypes.22

The rs2167270 genotyping findings indicated significant association. The GG genotype was more prevalent among patients, while the AG genotype was more prevalent among the controls. These results are supported by a study conducted among a South Indian population that pointed to a significant association with leptin gene expression, in which high leptin levels were observed with A alleles of the rs2167270 SNPs.23 In addition, multiple studies showed that homozygous carriers of the G allele had lower leptin levels compared to heterozygous or homozygous carriers for the A-allele.6,16,22,24 However, a study on a multi-ethnic Malaysian suburban population failed to find evidence supporting the effects of rs2167270 on leptin serum levels.25 Another study showed no significant associations with the occurrence of rheumatoid arthritis and development of cardiovascular events.26

These discrepancies may be due to inherent ethnic group differences as well as the differences in environmental conditions that might affect the gene expression profiles. Rs791620 and rs7799039 are located in the promoter region, while the rs2167270 SNP is located in the 5' untranslated region (UTR) of exon 1 of the LEP gene.16 and this, it might influence the level of leptin expression.27

In conclusion, the results of this study suggest the presence of a significant association between rs2167270 and AD, The GG allele was more frequently associated with patients, compared to the AA and AG alleles. In contrast, SNPs rs791620 and rs7799039 had no significant associations with AD.

Conflict of interest
The authors declare no conflict of interest.

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References
1. Hoffjan S, Stemmler S. Unravelling the complex genetic background of atopic dermatitis: from genetic association results towards novel therapeutic strategies. Arch Dermatol Res. 2015;307:659–70. doi:10.1007/s00403-015-1550-6.
2. Leung DY, Bieber T. Atopic dermatitis. Lancet 2003;361:151–60. doi:10.1016/S0140-6736(03)12193-9.
3. Novak N, Simon D. Atopic dermatitis – from new pathophysiologic insights to individualized therapy. Allergy 2011;66:830–9. doi:10.1111/j.1398-9995.2011.02571.x.
4. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Frontiers in endocrinology 2013;4:1–13. doi:10.3389/fendo.2013.00071.
5. Lago F, Dieguez C, Gómez-Reino J, Guallilo O. Adipokines as emerging mediators of immune response and inflammation. Nature Clinical Practice Rheumatology 2007;3:716–24. doi:10.1038/ncriorum00674.
6. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97. doi:10.1038/nri2921.
7. Shimabukuro M. Leptin Resistance and Lipolysis of White Adipose Tissue: An Implication to Ectopic Fat Disposition and Its Consequences. J Atheroscler Thromb 2017;24:1088–9. doi:10.5551/jat.ED083.
8. Nagel G, Koenig W, Rapp K, Wabitsch M, Zoellner I, Weiland SK. Associations of adipokines with asthma, rhino-conjunctivitis, and eczema in German schoolchildren. Pediatric Allergy and Immunology 2009;20:81–8. doi:10.1111/j.1399-3038.2008.00740.x.
9. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. Journal of Clinical Investigation 2003;111:1409. doi:10.1172/JCI200317490.
10. Faggioni R, Feingold KR, Grunfeld C. Leptin regulation of the immune response and the immunodeficiency of malnutrition. The FASEB Journal 2001;15:2565–71. doi:10.1096/fj.01-0431rev.
11. Saucillo DC, Gerriets VA, Sheng J, Rathmell JC, Maciver MA. Lack of association between LEP rs2167270 (19 G>A) polymorphism and disease susceptibility and cardiovascular disease in patients with rheumatoid arthritis. Clin Exp Rheumatol 2011;29:293–8.
12. Dossus L, Franceschi S, Biessy C, Navionis AS, Travis RC, Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Hager J, Clement K, Francke S, Dina C, Raison J, Lahlou N, Hager J, Clement K, Francke S, Dina C, Raison J, Lalhou N, Rich N, Pelloux V, Basdevant A, Guy-Grand B, et al. A polymorphism in the 5′ untranslated region of the human ob gene is associated with low leptin levels in South Indian population. Adipocyte 2015;4:135–40. doi:10.4049/jimmunol.1301158.
13. van Dielen FM, van’t Veer C, Schols AM, Soeters PB, Buurman WA, Greve JW. Increased leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. Int J Obes Relat Metab Disord 2001;25:1759–66. doi:10.1038/sjijo.0801825.
14. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 1998;394:897–901. doi:10.1038/30835.
15. Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P. A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. Horm Metab Res 2002;34:355–9. doi:10.1055/s-2002-33466.
16. Hager J, Clement K, Francke S, Dina C, Raison J, Lalhou N, Rich N, Pelloux V, Basdevant A, Guy-Grand B. A polymorphism in the 5′ untranslated region of the human ob gene is associated with low leptin levels. International journal of obesity 1998;22:200–5. doi:10.1038/sjijo.0800567.
17. Cieslak J, Bartz M, Stachowiak M, Skowronska B, Majewska KA, Harasymczuk J, Stankiewicz W, Fichna P, Switonski M. Effect of three common SNPs in 5′-flanking region of LEP and ADIPOQ genes on their expression in Polish obese children and adolescents. Mol Biol Rep 2012;39:3951–5. doi:10.1007/s11033-011-1174-7.
18. Kara N, Aydin F, Senturk N, Gunes S, Canturk MT, Bagci H, Bek Y, Turanli AY. Lack of association between the G-2548A polymorphism of the leptin gene and psoriasis in a Turkish population. Int J Dermatol 2007;46:1271–4. doi:10.1111/j.1365-4632.2007.03324.x.
19. Aydin F, Kara N, Senturk N, Gunes S, Canturk MT, Bagci H, Bek Y, Turanli AY. Lack of association between leptin G2548A gene polymorphism and Behcet’s disease. J Eur Acad Dermatol Venereol 2007;21:68–71. doi:10.1111/j.1468-3083.2006.01868.x.
20. Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F. Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight. Ann Hum Genet 2000;64:391–4. doi:10.1017/S0003480000008277.
21. Hinuy HM, Hirata MH, Forti N, Diament J, Sampaio MF, Armaganian D, Salazar LA, Hirata RD. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arq Bras Endocrinol Metabol 2008;52:611–6. doi:10.1590/S0040-27302008000000006.
22. Poitou C, Lacorte JM, Coupaye M, Bertrais S, Bedel J-F, Lafon N, Boullot J-L, Galan P, Borson-Chazot F, Basdevant A, et al. Relationship between Single Nucleotide Polymorphisms in Leptin, IL6 and Adiponectin Genes and their Circulating Product in Morbidly Obese Subjects before and after Gastric Banding Surgery. Obesity Surgery 2005;15:11–23. doi:10.1381/0960892052993431.
23. Dasgupta S, Salman M, Siddalingaiah LB, Lakshmi GL, Xavieor D, Sreenath J. Genetic variants in leptin: Determinants of obesity and leptin levels in South Indian population. Adipocyte 2015;4:135–40. doi:10.4161/21623945.2014.975538.
24. Hager J, Clement K, Francke S, Dina C, Raison J, Lalhou N, Rich N, Pelloux V, Basdevant A, Guy-Grand B, et al. A polymorphism in the 5′ untranslated region of the human ob gene is associated with low leptin levels. Int J Obes Relat Metab Disord 1998;22:200–5. doi:10.1038/sjijo.0800567.
25. Fan SH, Say YH. Leptin and leptin receptor gene polymorphisms and their association with plasma leptin levels and obesity in a multi-ethnic Malaysian suburban population. J Physiol Anthropol 2014;33:15. doi:10.1186/1880-6805-33-15.
26. Garcia-Bermudez M, Gonzalez-Juanatey C, Rodriguez-Rodriguez L, Vazquez-Rodriguez TR, Miranda-Filloy JA, Fernandez-Gutierrez B, Llorca J, Martin J, Gonzalez-Gay MA. Lack of association between LEP rs2167270 (19 G>A) polymorphism and disease susceptibility and cardiovascular disease in patients with rheumatoid arthritis. Clin Exp Rheumatol 2011;29:293–8.
27. Enns JE, Taylor CG, Zahradka P. Variations in Adipokine Genes AdipoQ, Lep, and LepR are Associated with Risk for Obesity-Related Metabolic Disease: The Modulatory Role of Gene-Nutrient Interactions. J Obes 2011;2011:168659. doi:10.1155/2011/168659.