A possible association between a dysfunctional skin barrier (filaggrin null-mutation status) and diabetes: a cross-sectional study

Jacob P Thyssen,1 Allan Linneberg,2 Berit C Carlsen,1 Jeanne D Johansen,1 Káre Engkilde,1 Torben Hansen,3,4 Flemming Pociot,5 Oluf Pedersen,3,6,7 Michael Meldgaard,8 Pal B Szecsi,8 Steen Stender,8 Torkil Menné1

ABSTRACT

Background: Filaggrin proteins are located in the skin and prevent epidermal water loss and impede the entry of micro-organisms, allergens and chemicals. Filaggrin null mutations are strongly associated with ichthyosis vulgaris and atopic dermatitis.

Objective: The authors aimed to investigate the association between filaggrin null mutations, atopic dermatitis and diabetes.

Design: A random sample of 3335 adults from the general population in Denmark was filaggrin-genotyped for R501X and 2282del4 null-mutations and questioned about atopic dermatitis and diabetes.

Results: In a crude data analysis, a positive association was detected between the filaggrin null genotype and, respectively, subjects from the general population who reported diabetes (p<0.04) and patients with established type 2 diabetes (p<0.073). Adjustment for age and gender resulted in significant associations for patients with type 2 diabetes (p=0.048) and subjects with self-reported diabetes (p=0.032).

Conclusions: Adult Danes with a filaggrin null genotype had a significantly increased prevalence of self-reported diabetes. This finding was replicated when an independent sample of Danish patients with type 2 diabetes was compared with participants from the general population who did not report diabetes and who had normal fasting plasma-glucose and glycated haemoglobin (HbA1c) levels.

INTRODUCTION

The outermost part of the skin acts as a barrier that protects against damage following exposure to, for example, mechanical insults, UV light, extreme temperatures, chemicals and micro-organisms. Furthermore, the skin has neuroendocrine and immune functions. Filaggrin proteins are crucial components of the terminal differentiation of the epidermis by aggregating keratin filaments. As such, filaggrin prevents epidermal water loss and impedes the entry of micro-organisms, allergens and chemicals.1

The filaggrin null genotype is observed in 10% of the general population.2,3 Loss of filaggrin expression disrupts the skin barrier and is strongly associated with ichthyosis vulgaris4 and atopic dermatitis.2 Furthermore, filaggrin null mutations are associated...
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with asthma and allergic rhinitis in combination with atopic dermatitis.\(^5\)\(^6\)

Few studies have investigated the skin barrier function in diabetics. An impaired state of stratum corneum hydration and a decreased skin surface lipid content in diabetic skin have been suggested in a mixed population of type 1 and 2 diabetics, and in diabetic mice.\(^7\)\(^8\) A case–control study found no difference in stratum corneum hydration and transepidermal water loss between diabetics and controls.\(^9\) A few studies have suggested the existence of an inverse association between atopic dermatitis and type 1 diabetes; a finding that may be explained by the T-helper (Th) cell 1 and 2 dichotomy.\(^10\)\(^11\) To our knowledge, no studies so far have investigated the possible association between diabetes type 2 and atopic dermatitis.

Filaggrin genotyping was recently performed in 3335 adults from the general population in Copenhagen.\(^3\) In our data analyses, we noticed a relatively high frequency of the null genotype in subjects who reported diabetes. We therefore hypothesised that a putative impairment of the skin barrier increases the propensity to low-grade inflammation, which again, in concert with other factors, could increase the risk of diabetes. In favour of such a mechanism, Bønnelykke et al recently found a filaggrin-associated pattern of atopic diseases in early childhood characterised by early onset of atopic dermatitis, early onset of asthma (independent of atopic dermatitis status) with severe exacerbations and later development of sensitisation.\(^12\) Thus, the existence of a specific endotype of asthma that is driven not by sensitisation but rather by skin-barrier dysfunction was suggested, since filaggrin is not expressed in airway mucosa.

Here, we investigated whether an association could be found between self-reported diabetes and, respectively, atopic dermatitis and filaggrin null mutation status. We included two independent study populations of patients who had type 1 or 2 diabetes, to determine whether a possible association could be replicated.

MATERIALS AND METHODS

Study populations

Ethic statement

The Ethical Committee of Copenhagen County approved the study (KA-20060011). Written and verbal consent was given by the participants to be included in the study and for their information to be stored in the hospital database and used for research.

Three independent Danish populations were included in the current study: (1) a random sample of adults from the general population in Copenhagen\(^13\); (2) patients with type 1 diabetes\(^14\); and (3) patients with type 2 diabetes.

Adults from the general population

Between June 2006 and May 2008, a cross-sectional study was performed in the general population in Copenhagen. A random sample of 7931 subjects aged 18–69 years was obtained from the Danish Central Personal Register. All were adults with Danish citizenship and born in Denmark. A total of 3471 (43.7%) subjects participated in a general health examination, and 3335 (96.1%) were filaggrin-genotyped for the 2282del4 and R501X mutations. The participation rate was higher among older age groups.\(^15\)

Patients with type 1 diabetes

A total of 104 patients diagnosed as having type 1 diabetes between 1981 and 2004 were randomly selected from a large incident cohort.\(^14\) All patients were diagnosed before age 18 years and according to WHO criteria. They were all positive for protein tyrosine phosphatase-like protein and/or glutamic acid decarboxylase 65 antibodies at diagnosis (0–3 months prior to the first insulin injection).

Patients with type 2 diabetes

A total of 774 (299 women, 475 men; age 65.2±11.4 years; BMI, 30.6±5.8 kg/m\(^2\)) unrelated patients diagnosed as having type 2 diabetes sampled randomly from the outpatient clinic at Steno Diabetes Center from 2005 to 2007 were included in the study.

Measurements (general population only)

Height and weight were measured in light indoor clothing and without shoes. Waist circumference was measured in the upright position midway between the iliac crest and the lower costal margin. Body mass index (BMI) was calculated in kg/m\(^2\). Blood-pressure measurements were performed after 5 min rest in the sitting position with a mercury sphygmomanometer. If the systolic or diastolic blood pressure exceeded 140 and 90 mm Hg, respectively, repeated measurements were made later during the health examination with the participant in a lying position. The lowest value was used.

Blood samples were drawn after a 12 h overnight fast. Fasting plasma glucose was analysed by a glucose oxidase method (Hitachi 912 system, Roche Diagnostics, Mannheim, Germany). Glactated hemoglobin (HbA1c) was analysed by the HPLC method (TOSOH, Minato, Japan). Serum insulin was measured using the AutoDELFIA insulin kit (Perkin-Elmer/Wallac, Turku, Finland). Fasting concentrations of total-, HDL- and LDL- cholesterol as well as triglyceride were measured using enzymatic colorimetric methods (Roche Molecular Biochemicals, Mannheim, Germany).\(^16\) The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate the degree of insulin resistance. The HOMA-IR index was estimated from fasting plasma glucose and fasting serum insulin concentrations using the following formula: HOMA-IR index=(fasting plasma glucose (mmol/l)·fasting serum insulin (mU/l))/22.5.\(^17\)

Filaggrin genotyping

Regions covering the mutations R501X and 2282del4 of the filaggrin gene were amplified from genomic DNA by PCR, and the obtained DNA fragments were hybridised
Logistic regression analysis using data from the general population only (table 3)

'Self-reported atopic dermatitis' was used as the independent variable, and gender, age, diabetes subgroup ('non-diabetes,' 'screen-detected diabetes' and 'self-reported diabetes') and filaggrin mutation status ('wildtype,' 'null-mutation') were explanatory variables. In a similar regression analysis, a test for interaction between diabetes subgroup and filaggrin mutation status was performed using a log-likelihood ratio test. This was carried out to determine whether the association between atopic dermatitis and diabetes depended significantly on filaggrin mutation status.

Characteristics were compared using the \( \chi^2 \) test. One-way ANOVA was used to compare means between different subgroups. Associations were expressed as ORs with 95% CIs. Data analyses were performed using SPSS for Windows (release 15.0).

RESULTS

A total of 3335 participants from the general population study (55.3% women), 104 patients diagnosed as having type 1 diabetes (40.4% women) and 774 patients diagnosed as having type 2 diabetes (38.5% women) were genotyped for the R501X and 2282del4 filaggrin null mutations. The observed genotype prevalences of both polymorphisms did not deviate significantly \((p>0.05)\) from the expected prevalences under the Hardy–Weinberg equilibrium assumption in any of these three populations.

The prevalence of self-reported diabetes was 4% in the general population (3.3% in women and 4.9% in men) (table 1). The vast majority of participants who reported diabetes were expected to suffer from type 2 diabetes. To add evidence to this assumption, we calculated the HOMA-IR in subjects without diabetes, those with screen-detected diabetes and those with self-reported diabetes (table 1). One-way ANOVA analysis revealed a statistically significant difference in the HOMA-IR mean between the three subgroups \((p<0.001)\). This supports the notion that type 2 diabetes was likely for the majority of cases with self-reported diabetes.

The prevalence of filaggrin mutations and atopic dermatitis was, respectively, 7.8% (95% CI 7.0 to 8.8) and diabetes status could depend on age. Another logistic regression analysis was performed with ‘diabetes’ as the dependent variable (‘self-reported diabetes’ and ‘screen-detected diabetes’) from the general population as well as patients from the ‘type 2 diabetes group’ were registered as diabetics, whereas patients with type 1 diabetes were regarded as missing data) and filaggrin mutation status (‘wildtype,’ ‘null-mutation’) and BMI \((<25,' 25–30,' >30'kg/m^2)\) as the independent variables. In this analysis, an interaction term between filaggrin mutation status and BMI was inserted to determine whether the association between filaggrin mutations and diabetes depended significantly on BMI.
Table 1  Characteristics of participants in the general population study stratified by gender, diabetes group and filaggrin mutation status

<table>
<thead>
<tr>
<th></th>
<th>All (n=3335)</th>
<th>Women (n=1844)</th>
<th>Men (n=1491)</th>
<th>Non-diabetes group (n=3136)</th>
<th>Screen-detected diabetes group (n=66)</th>
<th>Self-reported diabetes group (n=133)</th>
<th>Filaggrin wild type (n=3066)</th>
<th>Filaggrin null mutation (n=269)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (+2SD)</td>
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<tr>
<td>Mean age (years)</td>
<td>47.5 (26.0)</td>
<td>46.8 (26.2)</td>
<td>48.4 (25.8)</td>
<td>47.0 (26.0)</td>
<td>56.1 (21.6)</td>
<td>55.3 (20.4)</td>
<td>47.5 (26.1)</td>
<td>47.2 (25.8)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.7 (32.6)</td>
<td>69.9 (28.8)</td>
<td>85.1 (39.0)</td>
<td>76.17 (32.0)</td>
<td>83.3 (34.0)</td>
<td>86.0 (39.5)</td>
<td>76.7 (32.6)</td>
<td>76.8 (34.4)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 (0.19)</td>
<td>1.66 (0.12)</td>
<td>1.79 (0.13)</td>
<td>1.71 (18.4)</td>
<td>1.70 (17.2)</td>
<td>1.71 (19.1)</td>
<td>1.71 (18.4)</td>
<td>1.71 (18.6)</td>
</tr>
<tr>
<td>Waist (m)</td>
<td>0.88 (0.58)</td>
<td>0.83 (0.25)</td>
<td>0.95 (0.24)</td>
<td>87.9 (26.6)</td>
<td>97.4 (28.0)</td>
<td>100.4 (31.2)</td>
<td>88.5 (27.2)</td>
<td>89.4 (29.6)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.9 (9.3)</td>
<td>25.4 (10.0)</td>
<td>26.6 (8.2)</td>
<td>25.7 (9.06)</td>
<td>28.7 (11.2)</td>
<td>29.3 (11.6)</td>
<td>25.90 (10.4)</td>
<td>26.14 (10.4)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>130 (35)</td>
<td>127 (36)</td>
<td>134 (34)</td>
<td>129 (35)</td>
<td>141 (39)</td>
<td>137 (38)</td>
<td>130 (35)</td>
<td>130 (35)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>81 (21)</td>
<td>79 (20)</td>
<td>84 (21)</td>
<td>82 (21)</td>
<td>86 (23)</td>
<td>82 (18)</td>
<td>81 (21)</td>
<td>82 (21)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.23 (1.86)</td>
<td>5.13 (1.64)</td>
<td>5.36 (2.08)</td>
<td>5.10 (1.20)</td>
<td>7.37 (1.96)</td>
<td>7.24 (5.08)</td>
<td>5.22 (1.86)</td>
<td>5.25 (1.68)</td>
</tr>
<tr>
<td>Glycated haemoglobin (%)</td>
<td>5.41 (1.12)</td>
<td>5.38 (0.55)</td>
<td>5.45 (1.16)</td>
<td>5.35 (0.92)</td>
<td>6.17 (1.60)</td>
<td>6.49 (2.14)</td>
<td>5.41 (1.14)</td>
<td>5.44 (0.90)</td>
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<tr>
<td>Fasting blood cholesterol (mmol/l)</td>
<td>5.08 (2.04)</td>
<td>5.16 (2.06)</td>
<td>4.99 (1.00)</td>
<td>5.10 (2.00)</td>
<td>5.38 (2.50)</td>
<td>4.46 (1.86)</td>
<td>5.08 (2.04)</td>
<td>5.06 (1.96)</td>
</tr>
<tr>
<td>Fasting blood high-density lipoprotein cholesterol (mmol/l)</td>
<td>1.53 (0.86)</td>
<td>1.68 (0.85)</td>
<td>1.36 (0.72)</td>
<td>1.54 (0.86)</td>
<td>1.37 (0.73)</td>
<td>1.37 (0.81)</td>
<td>1.53 (0.85)</td>
<td>1.54 (0.86)</td>
</tr>
<tr>
<td>Fasting blood low-density lipoprotein cholesterol (mmol/l)</td>
<td>3.19 (1.94)</td>
<td>3.15 (1.90)</td>
<td>3.24 (1.99)</td>
<td>3.21 (1.92)</td>
<td>3.32 (2.57)</td>
<td>2.64 (1.74)</td>
<td>3.19 (1.94)</td>
<td>3.17 (1.98)</td>
</tr>
<tr>
<td>Fasting blood triglyceride (mmol/l)</td>
<td>1.29 (1.98)</td>
<td>1.16 (1.24)</td>
<td>1.46 (2.58)</td>
<td>1.27 (1.96)</td>
<td>1.76 (2.4)</td>
<td>1.51 (1.42)</td>
<td>1.30 (2.02)</td>
<td>1.24 (1.36)</td>
</tr>
<tr>
<td>Fasting blood insulin (pmol/l)</td>
<td>44.69 (75.61)</td>
<td>41.85 (63.22)</td>
<td>48.20 (88.24)</td>
<td>42.50 (65.04)</td>
<td>78.09 (99.66)</td>
<td>79.84 (177.50)</td>
<td>44.55 (76.34)</td>
<td>46.21 (67.38)</td>
</tr>
<tr>
<td>Homeostasis model assessment of insulin resistance*</td>
<td>10.84 (25.2)</td>
<td>9.91 (19.1)</td>
<td>12.00 (31.2)</td>
<td>9.8 (16.2)</td>
<td>26.3 (26.4)</td>
<td>27.9 (86.2)</td>
<td>10.81 (25.8)</td>
<td>11.16 (19.00)</td>
</tr>
<tr>
<td>Percentage (n/n_total)</td>
<td></td>
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<tr>
<td>Filaggrin null mutation</td>
<td>8.1 (269/3335)</td>
<td>8.5 (157/1844)</td>
<td>7.5 (112/1491)</td>
<td>7.8 (246/3136)</td>
<td>9.1 (6/66)</td>
<td>12.8 (17/133)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Atopic dermatitis†</td>
<td>10.0 (334/3335)</td>
<td>13.1 (242/1844)</td>
<td>6.2 (92/1491)</td>
<td>9.9 (310/3136)</td>
<td>9.1 (6/66)</td>
<td>13.5 (18/133)</td>
<td>9.1 (277/3066)</td>
<td>21.2 (57/269)</td>
</tr>
<tr>
<td>Self-reported diabetes‡</td>
<td>4.0 (123/3307)</td>
<td>3.3 (61/1830)</td>
<td>4.9 (72/1477)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.8 (116/3042)</td>
<td>6.4 (17/265)</td>
</tr>
</tbody>
</table>

The study was a cross-sectional study performed in the general population in Copenhagen during 2006–2008.

*(Fasting plasma glucose (mmol/l) × fasting serum insulin (mU/l))/22.5.
† Atopic dermatitis was defined by the UK Working Party’s diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria.18
‡ An affirmative answer to the question: ‘Have you ever been told that you suffered from diabetes?’ —, not done.

Filaggrin and diabetes

9.9% (95% CI 8.8 to 10.9) in non-diabetics, 9.1% (95% CI 3.9 to 18.8) and 9.1% (95% CI 2.1 to 16.1) in screening-detected diabetics, 12.8% (95% CI 8.0 to 19.6) and 13.5% (95% CI 7.6 to 19.3) in self-reported diabetics, 6.7% (95% CI 3.1 to 13.5) in patients with type 1 diabetes and 9.8% (95% CI 7.9 to 12.1) in patients with established type 2 diabetes. In a crude data analysis, no significant difference could be identified between non-diabetes and 9.9% (95% CI 0.91 to 2.48) and 1.11 (95% CI 0.66 to 1.88).

Finally, a logistic regression analysis restricted to the general population data was performed with ‘atopic dermatitis’ as the independent variable and revealed a positive and significant association between atopic dermatitis and diabetes. This means that stratification by age group was not necessary. However, the analysis revealed a positive and significant association between filaggrin null mutation status and, respectively, ‘self-reported diabetes’ and ‘type 2 diabetes’, when compared with non-diabetic controls from the general population.

Another logistic regression analysis was performed with ‘diabetes’ as the dependent variable and revealed a positive and significant association with filaggrin null genotype (table 2). An interaction term between filaggrin mutation status and BMI was significant (p=0.03); hence, filaggrin mutations were positively associated with diabetes in subjects with low BMI. Thus, when separate regression analyses were made with diabetes as the dependent variable and sex, age and filaggrin mutation as the explanatory variables in subjects with BMI<25, BMI=25–30 and BMI>30 kg/m², the OR for filaggrin mutation was, respectively, 2.08 (95% CI 1.15 to 3.76), 1.51 (95% CI 0.91 to 2.48) and 1.11 (95% CI 0.66 to 1.88).
Filaggrin and diabetes

Data from the general population study suggested that diabetes than in non-diabetics (tables 1 and 3). This finding was replicated when an independent sample of Danish patients with type 2 diabetes was filaggrin-genotyped and compared with participants from the general population who did not report diabetes and who had normal fasting plasma-glucose and HbA1c levels (table 2). No information about atopic dermatitis status was available from the sample of type 1 and 2 diabetes patients. For this reason, we could not determine whether filaggrin mutation status worked as a proxy for atopic dermatitis owing to the strong positive association between atopic dermatitis and filaggrin null mutation status or whether an association could be attributed to the filaggrin null genotype only. However, we showed that filaggrin null mutations did not increase the risk of diabetes in participants with atopic dermatitis as patients who visit their physician because of a chronic disorder are more likely to have been diagnosed with diabetes. This finding suggests that if filaggrin mutations truly increase the risk of developing diabetes, this might mainly be a factor in subjects with a low BMI. Thus, in obese subjects, factors other than the filaggrin genotype are of greater importance.

The vast majority of participants who reported diabetes were suspected of suffering from type 2 diabetes rather than the less prevalent type 1 diabetes. In support of this notion, a higher HOMA-IR was identified in participants who reported diabetes (table 1). A data analysis revealed that the prevalence of filaggrin null mutations was lower in patients with type 1 diabetes. This came as no surprise, since previous studies have identified an inverse association between type 1 diabetes and atopic dermatitis explained by the Th1/Th2 dichotomy. Thus, a Danish case–control study showed that among children who developed type 1 diabetes, the incidence of atopic dermatitis was significantly lower than in the controls before the onset of type 1 diabetes (OR=0.49; 95% CI 0.39 to 0.63). A large German case–control study showed that atopic dermatitis was less frequent in diabetic (13.3%) than in non-diabetic children (18.0%) and that atopic dermatitis was significantly associated with a reduced risk of type 1 diabetes (adjusted OR=0.71; 95% CI 0.53 to 0.96).

There were weaknesses in this study that should be addressed. First, genotyping was only performed for R501X and 2282del4, which cover approximately 85% of null mutations in the filaggrin gene among Caucasians. Second, the occurrence of atopic dermatitis was based on the UK Working Party’s Criteria, which have a sensitivity and specificity of 92% and 81%, respectively. Third, the question used to identify subjects with diabetes in the general population study has never been validated. Although this may have contributed additionally to misclassification in this study, table 1 shows that variables differed markedly between participants with and without self-reported diabetes. Missing information about serum C-peptid concentrations, insulin therapy and onset of diabetes might have been a better way to establish a diabetes diagnosis. Fourth, owing to small study populations, random error may have affected the study outcome. Thus, since this is the first study on this topic, we cannot exclude the possibility that the observed associations could be a type 1 error. Fifth, selection bias may have influenced the positive association between self-reported atopic dermatitis and type 2 diabetes, as patients who visit their physician because of a chronic disorder are more...
likely to undergo evaluation for other disorders. However, since patients with type 1 diabetes had a lower prevalence of filaggrin null mutations, and since such patients also regularly visit their physician, this may explain the positive association only to a small degree. Since the onset of atopic dermatitis occurs primarily in early childhood, and type 2 diabetes typically begins in adulthood, atopic dermatitis is likely to precede diabetes. Despite the presented weaknesses, general population studies are generally less biased than studies including patients and may be used to generate new hypotheses.

This study had a very novel finding and raises important questions, that is, is the increasing prevalence of type 2 diabetes and atopic dermatitis related? Could our findings be explained by an increased risk of diabetes following the use of topical corticosteroids in individuals with a disrupted skin barrier, despite such medicaments generally penetrating to a very small degree? Could repeated short-term oral corticosteroid therapy in some individuals with moderate to severe atopic dermatitis increase the risk of type 2 diabetes? Could chemicals, proteins and haptons that penetrate filaggrin-deficient skin more easily than normal skin in fact increase the propensity to develop low-grade inflammation, which again, in concert with other factors, could increase the risk of type 2 diabetes? These clinical questions are definitely important to explore further, since they may influence the diagnostic work-up and clinical course for patients with atopic dermatitis. Hence, further studies are obviously warranted to confirm or falsify our results, preferably prospective ones. A recent study is indirectly in favour of an association between atopic dermatitis and type 2 diabetes. In children with term births, maternal gestational diabetes was significantly associated with atopic dermatitis (OR=7.2; 95% CI 1.5 to 34.5) and allergen sensitisation (OR=5.7; 95% CI 1.2 to 28.0) in the offspring. If an association between atopic dermatitis/filaggrin null genotype and type 2 diabetes can be replicated in other studies, it might be considered to screen patients with atopic dermatitis for diabetes to a higher degree or limit the use of oral corticosteroid therapy. Since we believe this area should be explored further, we plan to reinvestigate the association between the filaggrin null mutations and diabetes in a 30-year follow-up study from the general population in Copenhagen and conduct cross-linkage studies of diabetes and atopic dermatitis databases.

Author affiliations:
1National Allergy Research Centre, Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Copenhagen, Denmark
2Research Centre for Prevention and Health, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark
3Hagedorn Research Institute and Steno Diabetes Center, Gentofte, Denmark
4Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
5Department of Clinical Experimental Research, Glostrup Research Institute, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark
6Faculty of Health Science, University of Aarhus, Aarhus, Denmark
7Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
8Department of Clinical Biochemistry, Copenhagen University Hospital Gentofte, Copenhagen, Denmark

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REFERENCES