Universidade de Aveiro Departamento de Química
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Armando José Cerejo Caseiro

Análise do Proteoma e Peptidoma Salivar na Diabetes Mellitus Tipo 1

Salivary Proteomics and Peptidomics of Type 1 Diabetes Mellitus

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Bioquímica, realizada sob a orientação científica do Doutor Francisco Manuel Lemos Amado, Professor associado da Universidade de Aveiro e do Doutor Rui Miguel Pinheiro Vitorino, Investigador auxiliar da Universidade de Aveiro.

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To my parents and sister

o júri

presidente

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palavras-chave

Saliva, diabetes mellitus tipo 1, proteoma, peptidoma, atividade proteolítica, complicações crónicas

resumo

A Diabetes Mellitus (DM) compreende um conjunto de desordens metabólicas comuns caracterizadas por hiperglicemia, que afeta diferentes órgãos do organismo. Ao longo do tempo, ocorrem danos microvasculares no glomérulo renal, retina e nervos periféricos, bem como doença macrovascular nas artérias. A composição da saliva também é afetada pela DM, com consequências na homeostasia oral. No entanto, o proteoma e o peptidoma salivar têm sido pouco explorados na DM tipo 1 e nas suas complicações crónicas. Tendo em conta o crescente interesse na saliva como fluido diagnóstico, o objetivo principal deste trabalho foi avaliar os eventos proteolíticos subjacentes à DM tipo 1 e às suas complicações microvasculares, bem como, caracterizar as alterações induzidas pela DM tipo 1 no proteoma e peptidoma salivar.

A DM tipo 1 e particularmente as complicações microvasculares associadas modulam o perfil proteolítico dos fluidos biológicos, com diferenças significativas de atividade observadas na urina e saliva, atribuídas principalmente ao complexo Metaloproteinase da Matriz (MMP)-9/lipocalina associada à gelatinase de neutrófilos, aminopeptidase N, azurocidina e calicreína 1. O aumento da atividade proteolítica observado na saliva total dos diabéticos resultou no aumento da percentagem de péptidos, principalmente de um número acrescido de fragmentos de colagénio do tipo I, refletindo possivelmente um estado inflamatório crónico dos tecidos orais e periodontais. O peptidoma também corrobora uma maior suscetibilidade das proteínas salivares, especificamente, das proteínas ricas em prolina básicas (bPRP) 1, bPRP2 e proteínas ricas em prolina ácidas (aPRP) à proteólise, evidenciando a geração de fragmentos de proteínas associadas à ligação a bactérias. A análise do proteoma salivar baseada em iTRAQ mostrou uma sobre-expressão de L-plastina, fator do adenocarcinoma do pâncreas e das proteínas S100-A8 e S100-A9, enfatizando a importância do sistema imune inato na patogénese da DM tipo 1 e das complicações microvasculares associadas. A análise integrada de todas as proteínas expressas diferencialmente entre os pacientes diabéticos com ou sem complicações microvasculares e indivíduos saudáveis foi realizada com o STRING, onde se observam três conjuntos funcionalmente ligados, um compreende a interação entre o colagénio tipo I, colagénio tipo II e MMP-9, um segundo conjunto envolve a MMP-2 e o colagénio de tipo I e um terceiro conjunto composto por proteínas salivares e inflamatórias. Estes conjuntos estão associados com as vias Kegg de interação recetor-matriz extracelular, de adesão focal e migração transendotelial dos leucócitos. Por outro lado, a análise do proteoma e peptidoma salivar destacou potenciais biomarcadores para o diagnóstico e prognóstico da DM tipo 1 e das suas complicações.

keywords

Saliva, type 1 diabetes mellitus, proteome, peptidome, proteolytic activity, chronic complications

abstract

Diabetes Mellitus (DM) comprises a set of common metabolic disorders that share the phenotype of hyperglycemia, which affect many different organ systems in the body. Over time, DM-specific microvascular disease in renal glomerulus, retina and peripheral nerves occurs, as well as macrovascular pathology in arteries. The composition of saliva is also affected by DM with consequences in the oral homeostasis; however, the salivary proteome and even more the peptidome has been quite unexplored in type 1 DM and related chronic complications. Taking into account the growing interest in saliva as diagnosis fluid, the main goal of this thesis was to disclose the proteolytic events underlying type 1 DM and related microvascular complications as well as to characterize DM-induced alterations in salivary proteome and peptidome.

Type 1 DM and particularly the associated microvascular complications modulates biofluids' proteolytic profile, with significant activity differences noticed for urine and saliva mainly attributed to Matrix Metalloproteinase (MMP)-9/neutrophil gelatinase-associated lipocalin complex, aminopeptidase N, azurocidin and kallikrein 1. The higher proteolytic activity noticed in whole saliva of diabetics leads to an increase in the percentage of peptides, mainly consisting of an augmented number of collagen type I fragments, possibly reflecting a chronic inflammatory state of oral and periodontal tissues. Moreover, peptidome data also support a diabetes-related higher susceptibility of salivary proteins, namely basic proline-rich protein (bPRP) 1, bPRP2 and acidic proline-rich proteins (aPRP) to proteolysis evidencing the generation of protein fragments associated with bacterial attachment. iTRAQ-based salivary proteome profiling evidenced an overexpression of L-plastin, pancreatic adenocarcinoma factor, protein S100-A8 and S100-A9, emphasizing the importance of the innate immune system in the pathogenesis of type 1 diabetes mellitus and related microvascular complications. The integrative analysis of all different expressed proteins performed with STRING shows three clusters functionally connected, one comprehending collagen types I and II interaction and MMP-9, a second involving MMP-2 and collagen type I, and a third cluster compreending salivary proteins and inflammatory proteins. These clusters are associated with the Kegg pathways extracellular matrix-receptor interaction, focal adhesion, and leukocyte transendothelial migration. In addition, the salivary proteome and peptidome analysis highlighted potential biomarkers for the diagnosis and prognosis of type 1 diabetes mellitus and related complications.

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IV - ABBREVIATIONS

1-DE	One-dimensional gel electrophoresis
1D-LC	One-dimensional liquid chromatography
2D-DIGE	Two-dimensional difference gel electrophoresis
2-DE	Two-dimensional gel electrophoresis
2D-LC	Two-dimensional liquid chromatography
AAI	Anti-insulin antibodies
ACN	Acetonitrile
AGEs	Advanced glycation end products
AMY1A	Alpha-amylase 1
apo	Apolipoprotein
aPRP	Salivary acidic proline-rich phosphoprotein 1/2
BPI	bactericidal/permeability-increasing protein-like 1
bPRP	Basic salivary proline-rich protein
CBB	Coomassie brilliant blue
CDK2	Cyclin-dependent kinase 2
CHAPS	3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate
CID	Collision-induced dissociation
CRP	C-reactive protein
CZE	Capillary zone electrophoresis
DM	Diabetes mellitus
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
FA	Formic acid
GAD65	anti-glutamic acid decarboxylase
GAPDH	Glyceraldehyde-3-phosphate
GDF9	Growth differentiation factor-9
GeLC	In-gel tryptic digestion followed by liquid chromatography
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein
HILIC	Hidrophilic interaction chromatography
HLA	Histocompatibility leukocyte antigen
HNC	Head and neck cancer
HPLC	High-performance liquid chromatography
ICA	Islet cell antibody
ICAT	Isotope coded affinity tags
IEF	Isoelectric focusing
IgG	Immunoglobulin G
IL	Interleukin
iTRAQ	Isobaric tagging for relative and absolute protein quantification
kDa	Kilodalton
LC	Liquid chromatography

LCAT	Lecithin-cholesterol acyltransferase
LRP1	Low density lipoprotein receptor-related protein 1
MALDI	Matrix-assisted laser desorption/ionization
MBL	Mannan-binding lectin
MMP	Matrix metalloproteinase
MMTS	S-methyl methanethiosulfonate
MRP8/14	Myeloid-Related Protein-8/14 complex
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MUC	Mucin
MudPIT	Mutidimensional protein identification technology
MW	Molecular weight
N	Nephropathy
NF-kB	Nuclear factor-κB
NGAL	Neutrophil gelatinase-associated lipocalin
NSGP	Nonsalivary gland secreted peptides
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PAUF	Pancreatic adenocarcinoma upregulated factor
PFF	Peptide fragment fingerprinting
p*I*	Isoelectric point
PIgR	Polymeric immunoglobulin receptor
PLS-DA	Partial least squares - discriminant analysis
PLUNC	palate, lung and nasal epithelium associated protein
PMF	Peptide mass fingerprinting
PMSF	Phenylmethylsulfonyl fluoride
PRDX3	Thioredoxin-dependent peroxide reductase
PRG	Proline-rich glycoprotein
PROL4	Proline-rich protein 4
PRPs	Proline-rich proteins
PTMs	Post-translational modifications
R	Retinopathy
RIF1	Rap1-interacting-factor-1
RP-RP	Two-dimensional reverse-phase
SCX	Strong cation exchange
SDS	Sodium dodecyl sulphate
SGP	Salivary gland secreted peptides
SILAC	Stable isotope labeling by amino acids in cell culture
SILAP	Stable isotope labeled proteome
SLPI	Secretory leukocyte protease inhibitor
SMR3B	Submaxillary gland androgen regulated protein 3B
SOD2	Superoxide dismutase 2
SS	Sjögren's syndrome
STD	Standard deviation

T1D	Type 1 diabetic
TBS	Tris buffered saline
TBS-T	TBS-Tween
TCEP	Tris(2-carboxyethyl) phosphine
TEA	Triethylamine
TEAB	Triethyl ammonium bicarbonate
TFA	Trifluoroacetic acid
TLR	Toll-like receptor
TOF	Time-of-flight
TP53	Tumor protein 53
Tris	Tris(hydroxymethyl) aminomethane
UV	Ultraviolet
VCAM	Vascular cell adhesion molecule
WHO	World health organization
ZnT8	Anti-zinc transporter 8
α-CHCA	α-cyano-4-hydroxycinnamic acid

CHAPTER I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1. Diabetes Mellitus: definition, epidemiologics and diagnosis

Diabetes Mellitus (DM) comprises a set of common metabolic disorders that share the phenotype of hyperglycemia with disturbances of carbohydrates, fat and protein metabolism (WHO 1999; Assoc 2012). There are distinct types of DM caused by a complex interplay of genetic factors, environmental and lifestyle. According to the etiology of DM, the causes that contribute to hyperglycemia may include reduction in insulin secretion, resistance to its action, or both (Deshpande *et al.* 2008; Guideline 2009). The majority of DM cases belong to two etiopathogenic categories, designated as type 1 and type 2. Diabetes Mellitus can also occur during pregnancy, classified as gestational diabetes, corresponding to any degree of glucose intolerance, documented for the first time during pregnancy (WHO 1999; Assoc 2012). There are other specific types of diabetes, caused by genetic defects that affect the function of pancreatic β cells or insulin, pancreas disease or drugs (Deshpande *et al.* 2008; Fraser 2009; Assoc 2012; Ghazarian *et al.* 2012).

In the first category, type 1 DM, the origin of pathology is an absolute deficiency of insulin secretion, resulting from pancreatic β cells destruction, being the insulin therapy essential to ensure the survival of patients. In most cases, the destruction of the cells is produced by an autoimmune mechanism and is designated as autoimmune type 1 DM (Ghazarian *et al.* 2012). However, in some cases is not possible to document the associated etiology, being classified as idiopathic type 1 DM (Deshpande *et al.* 2008; Guideline 2009).

In the more prevalent category, type 2 DM, the combination of resistance to insulin action with an inadequate secretory compensatory response is the cause of pathology (Assoc 2012). In type 2 DM, the level of hyperglycemia is high enough to cause pathological functional changes in various target tissues and can occur over a long period of time before disease's detection and in the absence of clinical symptoms (WHO 1999; Assoc 2012). During this asymptomatic period, it is possible to monitor changes in carbohydrates metabolism by testing the fasting plasma glucose or after an overload with oral glucose (Mayor 2007; Deshpande *et al.* 2008; Guideline 2009; Surampudi *et al.* 2009).

The prevalence of DM varies in the different regions of the world, though with a gradual growth, mainly in the age group over 45 years (Deshpande *et al.* 2008; Chan *et al.* 2009;

Grant *et al.* 2009; Borchers *et al.* 2010). In the last two decades it was observed an increased incidence of diabetes, which is expected to continue in the next years due to the rise of obesity, physical inactivity and aging of the population (Grant *et al.* 2009; Whiting *et al.* 2011; Hummel *et al.* 2012; Lam *et al.* 2012). According to the International Diabetes Federation, in 2011 approximately 366 million people in the world had diabetes, being predicted to increase to about 552 million in 2030 (Whiting *et al.* 2011). The prevalence of diabetes in the Portuguese population was estimated to be 11.7% in 2009 by the study PREVADIAB-2009 (Gardete-Correia *et al.* 2010). This study points to the existence of 905,035 diabetic subjects with ages between 20 and 79 years in Portugal, with significant differences between men and women, with a prevalence of 14.2% and 9.5%, respectively (Gardete-Correia *et al.* 2010). Moreover, the percentage of undiagnosed diabetes was estimated to be approximately 5.1% in 2009 (Gardete-Correia *et al.* 2010), further supporting the conviction of a growing trend of the prevalence of diabetes in Portugal (Rodrigues *et al.* 1992; Macedo *et al.* 2003; Duarte-Ramos *et al.* 2006).

The clinical diagnosis of diabetes is often suggested by the presence of symptoms such as: polydipsia and polyuria, recurrent infections, unexplained weight loss and, in severe cases, lethargy and coma (Kasper *et al.* 2005). In patients with classic symptoms of hyperglycemia or hyperglycemic crisis, an unequivocal hyperglycemia establishes the diagnosis (random plasma glucose above 200 mg/dl) (Kasper *et al.* 2005; Assoc 2012). According to the World Health Organization there are other three criteria for the diagnosis of DM: glycated hemoglobin (HbA1c) ≥ 6.5% performed in a certified laboratory using a standardized assay; fasting plasma glucose ≥ 126 mg/dl with a fasting of at least 8 hours or a 2-h plasma glucose ≥ 200mg during an oral glucose tolerance test. However, in the absence of unequivocal hyperglycemia, these criteria must be confirmed by repeated testing (Assoc 2012).

In terms of chronic glycemia evaluation, the widely used marker is HbA1c, reflecting average blood glucose levels over a 2- to 3-month period of time (Assoc 2012). The advantages of HbA1c are not limited to the convenience of non-fasting, but essentially as a standard biomarker in the adequacy of glycemic management, presenting a well correlation of test values with the development of both microvascular and, to a lesser extent, macrovascular complications (Assoc 2012).

In order to effectively treat DM, it is also important the differential diagnosis of type 1 *versus* type 2 (Assoc 2012). In this sense, biomarkers like C-peptide levels, islet

autoantibodies and genetic tests contribute to a reliable clinical diagnosis (Assoc 2012; Nokoff *et al.* 2012), The correct classification of the disease also helps to identify the possible etiologies and to develop specific prevention for type 1DM (Rewers 2012).

1.1. Type 1 DM: pathogenesis, clinical onset and related chronic complications

Type 1 DM, formerly known as juvenile-onset or insulin-dependent diabetes, comprises about 5-10% of the subjects with diabetes and results from an autoimmune destruction of pancreatic β cells (van Belle *et al.* 2011; Assoc 2012). This serious condition is the most common metabolic disease in children (Maahs *et al.* 2010; Gan *et al.* 2012). Autoantibodies are currently the best biomarkers of type 1 DM (Dang *et al.* 2011) and approximately 85-90% of individuals at diagnosis present one or more markers of pancreatic islets β-cells autoimmune destruction. These markers include anti-insulin autoantibodies (AAI), anti-islets of Langerhans (Islet Cell Antibody-ICA), anti-glutamic acid decarboxylase (GAD65), anti-zinc transporter 8 (ZnT8) and anti-tyrosine phosphatases insulinoma antigen IA-2 and IA-2β (Wilkin *et al.* 1985; Goldstein *et al.* 2003; Orban *et al.* 2009; Tsirogianni *et al.* 2009; Sorensen *et al.* 2012). The rate of pancreatic β-cells destruction is quite variable, being faster in newborns and children and slower in adults (Assoc 2012).

Type 1 DM is associated with genetic as well as environmental factors that could play an important role in triggering β-cells autoimmunity (Assoc 2012; Gan *et al.* 2012). Studies carried out in different ethnic groups suggest that type 1 DM-related genetic susceptibility is associated with the presence of certain major histocompatibility antigens (Histocompatibility Leukocyte Antigen - HLA). HLA-DR3 and HLA-DR4 haplotypes were detected in 90 to 95% of European Caucasians subjects with type 1 DM (Wang *et al.* 2007; Orban *et al.* 2009; Qu *et al.* 2009; van Belle *et al.* 2011). Nevertheless, only few subjects with apparent genetic predisposition really end up getting type 1 DM and the increase prevalence noticed in several developed countries cannot be explained by genetics. Given the incapacity of genetic factors to predict alone the development of the disease, environmental factors such as viral infections, diet, cow milk during childhood, food toxins, drugs, stress or vitamin D deficiency are potentially involved (Akerblom *et al.* 1998; Gan *et al.* 2012; Phlips *et al.* 2012). There are still some forms of type 1 DM with unknown etiology, being more prevalent in Africa and Asia. In these cases, patients have a permanent insulinopenia and tendency to ketoacidosis, not showing, however, any

evidence of an autoimmune process, (McLarty *et al.* 1990; Deshpande *et al.* 2008; Assoc 2012).

The most common clinical manifestation of the disease is ketoacidosis, especially in children and adolescents. Some patients present modest fasting hyperglycemia that rapidly evolves to severe hyperglycemia and ketoacidosis in the presence of a stress situation like an infection (Assoc 2012). Certain individuals, mostly adults, keep a residual β-cell function for many years, enough to prevent episodes of ketoacidosis. In a later stage of the disease, with the reduction of insulin secretion confirmed by low or undetectable plasma levels of C-peptide, these patients probably will become insulin dependent and at risk for ketoacidosis (Assoc 2012; Sorensen *et al.* 2012).

Type 1 DM can affect many different organ systems in the body and, over time, is characterized by the development of diabetes-specific microvascular pathology in renal glomerulus, retina and peripheral nerves, as well as macrovascular pathology in arteries (Deshpande *et al.* 2008). As consequence of the microvascular damage, diabetes is the major cause of end-stage renal disease and new blindness in people with age between 20-74 years (Pyram *et al.* 2012). The diabetic neuropathy affects over 60% of subjects leading to urinary incontinence, gastroparesis, nocturnal diarrhea and erectile dysfunction (Sperling 2003). The combination of extremity arterial disease and neuropathy in diabetes accounts for 50% of all nontraumatic amputations in the United States (Sperling 2003). Subjects with diabetes are in a greater risk for developing cardiovascular complications and their life expectancy is about 7-10 years shorter (Ruderman *et al.* 1992; Brown *et al.* 2010). Several epidemiological studies in type 1 DM show a strong relationship, continuous but not linear, between blood glucose levels and the risk of chronic complications development (Shamoon *et al.* 1993; Brown *et al.* 2010).

Diabetic retinopathy, a major microvascular complication, is a multifactorial disease of the retina that presents an extremely complex pathogenesis, involving several types of cells and molecules (Ola *et al.* 2012; Zhang *et al.* 2012). Diabetic retinopathy is observed in approximately 13% of the patients with less than 5 years of disease, increasing up to 90% after 10-15 years of disease (Sperling 2003). Diabetic nephropathy is the leading cause of kidney disease, affecting approximately 40% of diabetic patients (Sperling 2003). This pathology has been categorized into stages based on the values of urinary albumin excretion: microalbuminuria and macroalbuminuria (Gross *et al.* 2005). Urinary albumin excretion is the main employed clinical marker of diabetic nephropathy stage and progression and play a key role in the long-term evaluation of diabetic patients (Gross *et*

al. 2005). Although microalbuminuria has been recognized as an early indicator of diabetic nephropathy, it is not a completely reliable index of renal histology (Sperling 2003). The presence of albuminuria might not be always indicative of diabetic nephropathy and some patients presenting significant glomerular mesangial matrix expansion in the absence of albuminuria (Messent *et al.* 1992; Caramori *et al.* 2000; Molitch *et al.* 2004). Moreover, urinary albumin excretion may be reversibly affected by glycemic control, hypertension, exercise, congestive heart failure or urinary tract infections (Caramori *et al.* 2000).

1.2. Proteomics and peptidomics of biofluids for DM characterization

In recent years there has been a huge development in the field of protein identification and characterization in complex biological samples. The platforms for proteome and peptidome analysis have advanced over the last few years, driven by the technologic development, being translated in a substantial increase in the number of studies focused in the proteome/peptidome analysis of biofluids (Schrader *et al.* 2001; Ramstrom *et al.* 2004; Crameri 2005; Soloviev *et al.* 2005; Albalat *et al.* 2011). While the term proteomics has been used to state the analysis of proteins expressed by organisms, the relatively recent concept, peptidomics, defines the comprehensive analysis of small peptides and polypeptides of a biological sample (peptidome), unexplored by proteomics analysis (Baggerman *et al.* 2004; Amado *et al.* 2005; Menschaert *et al.* 2010; Gao *et al.* 2011).

The application of proteomics and peptidomics on clinically relevant problems (*e.g.* cancer, diabetes and kidney disease) is commonly called clinical proteomics/peptidomics. The continuous development of mass spectrometry (MS)-based thecnologies and platforms that enable a better characterization of proteome and peptidome greatly contributed to an increase in biomarker discovery. Indeed, in the last 15 years over a thousand biomarkers have been published (Rao *et al.* 2007; Matt *et al.* 2008; Borrebaeck 2012), which reflect the consequences of pathophysiological conditions in the production and metabolism of proteins and peptides in human cells and tissues that are detectable in bodily fluids (Schrader *et al.* 2001). The great diversity of peptides with clinical interest in living systems has led to the rapid development of peptidomics, contributing to the discovery of new therapeutic targets and biomarkers. Specific peptide classes as antimicrobial ones or related to metabolic diseases such as diabetes and obesity are increasingly in focus (Brockmann *et al.* 2009; Brown *et al.* 2009; Quintana *et al.* 2009; Wei *et al.* 2009; Westman-Brinkmalm *et al.* 2009). The main objective of clinical-omics is convert the information provided by massive protein/peptide profiling in clinical aplications, with expected profit in early diagnosis of disease, monitorization of treatments,

identification of novel terapeutic targets and prediction of disease outcome or response to treament (Celis *et al.* 2008; Rodriguez-Suarez *et al.* 2012). A valuable advantage of clinical proteomics is the possibility of providing non-invasive biomarkers by the analysis of easily accessible body fluids such as blood, urine or saliva (Apweiler *et al.* 2009; Rodriguez-Suarez *et al.* 2012). The search for subclinical disease biomarkers as well as for chronic complications risk prediction driven by proteomic approaches resulted in the identification of more than 300 up- or downregulated distinct proteins in different fluids from DM patients (Kuzuya *et al.* 2002; Padrao *et al.* 2012).

Serum or plasma is considered the first choice of specimen given its fullness of biological information and relatively easy collection. Nevertheless, the hight complexity of serum and plasma samples, as well as the large dynamic range of protein concentrations require sample pretreatment in order to explore the low abundant proteins (Zhi *et al.* 2010). In spite of these analytical challenges, proteomics of blood-derived fluids resulted in the identification of many type 1 DM potential biomarkers (Molitch *et al.* 2004; Metz *et al.* 2008; McGuire *et al.* 2010; Overgaard *et al.* 2010; Overgaard *et al.* 2010; Vitorino *et al.* 2010; Rewers 2012). However, as recently reviewed (Padrao *et al.* 2012), from the identified DM-modulated proteins on plasma samples, only apolipoprotein (apo)-1, apoA-2, apoB-100, apoC-1, apoC-3, apoE, beta-2-glycoprotein 1 and clusterin were validated as biomarkers for type 1 diabetes and related complications (Padrao *et al.* 2012). Interstingly, serum protein profiling retrieved, as potential specific markers to type 1 DM, adiponectin, amyloid A protein, haptoglobin, insulin-like growth factor binding protein 2, myeloperoxidase, transforming growth factor beta. Several proteins like C-Reactive Protein (CRP), alpha-2-macroglobulin, coagulation factor IX, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), myocilin, peroxiredoxin-2, thyroxine-binding globulin and von Willebrand factor were pointed as potential biomarkers for the both types of DM (Padrao *et al.* 2012).

Other studies focused on the prediction of type 1 DM-related chronic complications like nephropathy and retinopathy were performed in blood-derived samples. The search for new biomarkers to predict the risk of diabetic nephropathy conducted by Overgaard *et al.* (2010) using a SELDI-TOF-MS approach resulted in the identification of 16 peak clusters, assigned as transthyretin, apolipoprotein A1, apolipoprotein C1 and cystatin C. The same cohort analyzed with an iTRAQ-based approach enabled the identification of 112 proteins, with apoA-2, B, C3, D and E as key nodes in the top-rated ingenuity network, after interactive pathway analysis. These biomarkers have potential to be used as progression

clinical markers in the treatment and monitoring of diabetic nephropathy, but further validation with a longitudinal study is needed (Overgaard *et al.* 2010).

A recent comprehensive study integrating multiple state-of-the-art proteomic technologies was developed by Zhi *et al.* (2011) with the aim of systematically assess the proteome profile associated with type 1 DM. This work enabled the identification of 21 differentially expressed serum proteins between diabetic subjects and controls, implicated in inflammation, oxidation, metabolic regulation, and autoimmunity. From these proteins, adiponectin, insulin-like growth factor binding protein 2, serum amyloid protein A, CRP, myeloperoxidase, and transforming growth factor beta were further validated by two different imunoassays in a large data set (Zhi *et al.* 2011). Ahn *et al.* (2006), using immunoproteomic technology, identified 20 human retinal protein spots that were antigenic in patients with diabetic retinopathy. The observed autoantibodies to aldolase C, creatine kinase B, phosphoglycerate kinase 1 and carbonic anhydrase II were specific to subjects with diabetic retinopathy. More recently, Sasongko *et al.* (2011) used a cross-sectional study of 224 diabetic patients to assess the serum levels of apoA-1 and apoB and verified that the content of apoA-1 and the apoB-to-apoA-1 ratio were associated with diabetic retinopathy severity, independently of sex, age and risk factors.

In biomedical research, urinary proteomics is performed with the aim of identify new tools for diagnosis and monitoring of kidney and non-kidney diseases (Merchant *et al.* 2010; Lapolla *et al.* 2011; Padrao *et al.* 2012). The large array of proteins present in urine, reflect not only kidney and urogenital tract physiology as well as systemic changes, given the contribution of plasma proteins to urine through glomerular filtration or leakage and tubular secretion (Lescuyer *et al.* 2007; Hubel *et al.* 2011). In the last years, several biomarkers for urinary tract, blader and prostate cancer, as well as predictors of renal function and diabetic nephropathy progression were identified in urine (Merchant *et al.* 2009; Hubel *et al.* 2011). The non-invasive collection, the reduced proteolytic activity in comparison with blood and the possibility of obtain large quantities of sample made urine an atractive fluid for clinical proteomics (Hubel *et al.* 2011). However, its non-homogeneous composition, the protein concentration dependance on disease state and the presence of high concentrations of urea and salts limit the proteomic analysis of urine proteome (Lescuyer *et al.* 2007; Hubel *et al.* 2011).

The urinary proteome in DM has been studied using different proteomic approaches and several biomarkers were identified (*e.g.* orosomucoid, beta-2-microglobulin, epithelial-cadherin, IgG, ubiquitin, zinc-alpha-2-glycoprotein, albumin, haptoglobin, retinol binding

protein 4, transthyretin and alpha-1-antitrypsin) associated with distinct molecular functions like immune, cell adhesion, response to stimulus, transport and signal pathways (Padrao *et al.* 2012). Although most of the studies have been performed in type 2 DM, some research works have been conducted in subjects with type 1 DM (Rossing *et al.* 2008; Merchant *et al.* 2009; Thrailkill *et al.* 2009; Schlatzer *et al.* 2012; Soggiu *et al.* 2012; Zurbig *et al.* 2012). With the purpose of identify distinctive molecular features in urine samples that might correlate to type 1 DM complications, Soggiu *et al.* (2012) analyzed diabetic patients with normo- and microalbuminuria, using high resolution 2-DE and LC-MS/MS analysis. Data obtained revealed lower levels of Tamm-Horsfall urinary glycoprotein, apoA-1, apoE, alpha-2 thiol proteinase inhibitor and human CD59 and higher protein representations for alpha-1-microglobulin, zinc-alpha-2-glycoprotein, alpha-1B-glycoprotein and retinol binding protein 4, providing evidences of urine proteins potentially exploitable as putative prognostic biomarkers (Soggiu *et al.* 2012).

An attempt to identify urinary proteins implicated in proteinuria pathophysiology was developed by Thrailkill *et al.* (2009). An SDS-PAGE hyphenated with LC–MS/MS platform was used to compare the urine proteome from healthy nondiabetic individuals, subjects with type 1 DM with normoalbuminuria and subjects with type 1 DM and microalbuminuria. The significantly increase of megalin and cubilin, two endocytic receptors involved in the reuptake of filtered albumin in kidney proximal tubule cells, seem to contribute to the albuminuria detected in these patients (Thrailkill *et al.* 2009). With the same purpose, Merchant *et al.* (2009) conducted a LC-MALDI-TOF approach focused on DM-related renal function decline. A decrease in the fragments of collagen alpha 1(IV) and alpha 1(V) and tenascin-X and an increase of fragments of inositol pentakisphosphate 2-kinase, zona occludens 3, and FAT tumor suppressor 2 was observed. The higher expression of inositol pentakisphosphate 2-kinase was confirmed in renal biopsies from type 1 DM patients with early nephropathy, being suggested as predictor of diabetic nephropathy progression (Merchant *et al.* 2009).

Rossing *et al.* (2008) developed a capillary zone electrophoresis (CZE)-MS approach involving 305 individuals to discriminate urinary biomarkers for diabetes, diabetic nephropathy and nondiabetic proteinuric renal diseases. A selection of 40 biomarkers, including collagen type I and uromodulin fragments, distinguished subjects with diabetes from healthy individuals with 89% sensitivity and 91% specificity. Furthermore, a panel of 65 urinary biomarkers allowed the discrimination of patients with diabetic nephropathy from patients with other chronic renal diseases with 81% sensitivity and 91% specificity.

Other fluids are also attractive for biomarker discovery namelly ocular ones (tears, aqueous humor and vitreous), considering the minimally invasive procedure for sample collection and the potential clinical application to ocular diseases or other conditions like diabetic retinopathy (Garcia-Ramirez *et al.* 2007; Acera *et al.* 2012; Kim *et al.* 2012; Srinivasan *et al.* 2012). In the last years several promisor biomarkers for diabetic retinopathy and potential therapeutic targets like apoA-1, apoH, complement C3, complement C4b, complement factor B, retinol-binding protein 3 and zinc-alpha-2-glycoprotein were identified and validated in ocular fluids from type 1 DM subjects (Padrao *et al.* 2012). ApoA-1 and apoH proteins were identified by 2D-DIGE-MS/MS and validated with western blot in vitreous fluid as biomarkers of proliferative diabetic retinopathy (Gao *et al.* 2008; Simo *et al.* 2008). Tears have also been used to extend the knowledge about the pathophysiology of retinopathy in type 1 DM (Csosz *et al.* 2012; Kim *et al.* 2012). Kim *et al.* (2012) and Csosz *et al.* (2012) investigated the protein profile in subjects with non proliferative retinopathy, with DM without retinopathy and healthy volunteers and reported disease-related upregulated proteins Dj-1 protein and beta-2-microglobulin.

In the latest years, saliva has attracted widespread interest as a diagnostic fluid (Greabu *et al.* 2009; Pink *et al.* 2009; Farnaud *et al.* 2010; Bartoszewicz *et al.* 2011; Castagnola *et al.* 2011; Malamud 2011; Pfaffe *et al.* 2011). The recent advancements in salivary proteomics hold special promise in the disclose of novel biomarkers and therapeutic targets (Hardt *et al.* 2005; Al-Tarawneh *et al.* 2011). Indeed, the application of proteomic technologies pave the way to new potential salivary biomarkers of oral and systemic diseases as dental and gingival pathology, salivary gland disease, Sjögren syndrome, diabetes, head and neck carcinoma, breast and gastric cancers, sclerosis and psychiatric and neurological diseases (Greabu *et al.* 2009; Pink *et al.* 2009; Rao *et al.* 2009; Cabras *et al.* 2010; Al-Tarawneh *et al.* 2011; Castagnola *et al.* 2011; Border *et al.* 2012). However, little emphasis has been given to salivary proteome and peptidome analysis in DM, especially in type 1 (Hirtz *et al.* 2006; Cabras *et al.* 2010). The growing importance of quantitative proteomic approaches for clinical applications has been increasingly recognized, aiming to provide the screening of non-physiological levels of certain proteins and/or peptides that might reflect pathological conditions (Castagnola *et al.* 2011). No other studies are known that quantitatively evaluate the saliva proteome and peptidome changes related with type 1 DM and related chronic complications to this pathological condition.

1.3. Saliva as diagnostic fluid

Saliva is a singular fluid and the interest in its use as auxiliary means of diagnosis has grown exponentially in recent years. An increasing number of drugs, hormones and antibodies can be measured with reliability in saliva (Dodds *et al.* 2005). Nowadays, the analysis of saliva is used in the diagnosis of oral diseases (Siudikiene *et al.* 2008; Levine 2011), infections, cancer (Dowling *et al.* 2008; Schaaij-Visser *et al.* 2010), hereditary (Cabras *et al.* 2010), autoimmune (Giusti *et al.* 2007; Baldini *et al.* 2008) and endocrine diseases (Sundsten *et al.* 2009; Overgaard *et al.* 2010).

Saliva is easy to collect and its non-invasive nature makes it an attractive alternative to blood tests. Compared with blood sampling, saliva collection reduces the discomfort, anxiety and simplifies the collection of samples in series. The analysis of saliva can offers an approach with a good cost-effectiveness to screening diseases in large populations, as well as use in children and the elderly, where the blood harvest presents further complications. Additionally, saliva tests are more secure than those in blood, relatively to contamination risk of the laboratory professionals (Kaufman *et al.* 2002; Streckfus *et al.* 2002; Castagnola *et al.* 2011).

1.3.1. Saliva's protein profile vs. physiological role

Saliva is a unique complex mixture of glycoproteins, enzymes, hormones and growth factors that plays important physiological functions (Figure 1) (Ghafouri *et al.* 2003; Aps *et al.* 2005; Walz *et al.* 2006). The protective properties of saliva include lubrication functions, initiation of digestion, anti-microbial protection, secretion of antibodies, protection against mechanical and chemical properties and hydration of the oral cavity, oropharynx and esophagus mucous membranes (Aps *et al.* 2005; Farnaud *et al.* 2010). The physiological relevance of saliva is unambiguously evidenced in individuals with xerostomy, resulting in the decreased salivary flow, severe tooth decay, opportunistic microorganisms infection and oral pain (Gorr *et al.* 2005).

Saliva is secreted by salivary glands, composed by three major pairs (submandibular, parotid and sublingual) (Figure 2), numerous small glands distributed by the tongue, palate, oral and labial mucosa (Aps *et al.* 2005; Sun *et al.* 2008). These glands produce a high volume of saliva in relation to its size, being the maximum amount produced approximately 1 ml/min/g of glandular tissue. Globally, salivary glands are responsible for daily production of about 800 to 1500 ml of saliva (Ellis 1991; Aps *et al.* 2005; Melvin *et al.* 2005; Moore *et al.* 2010).

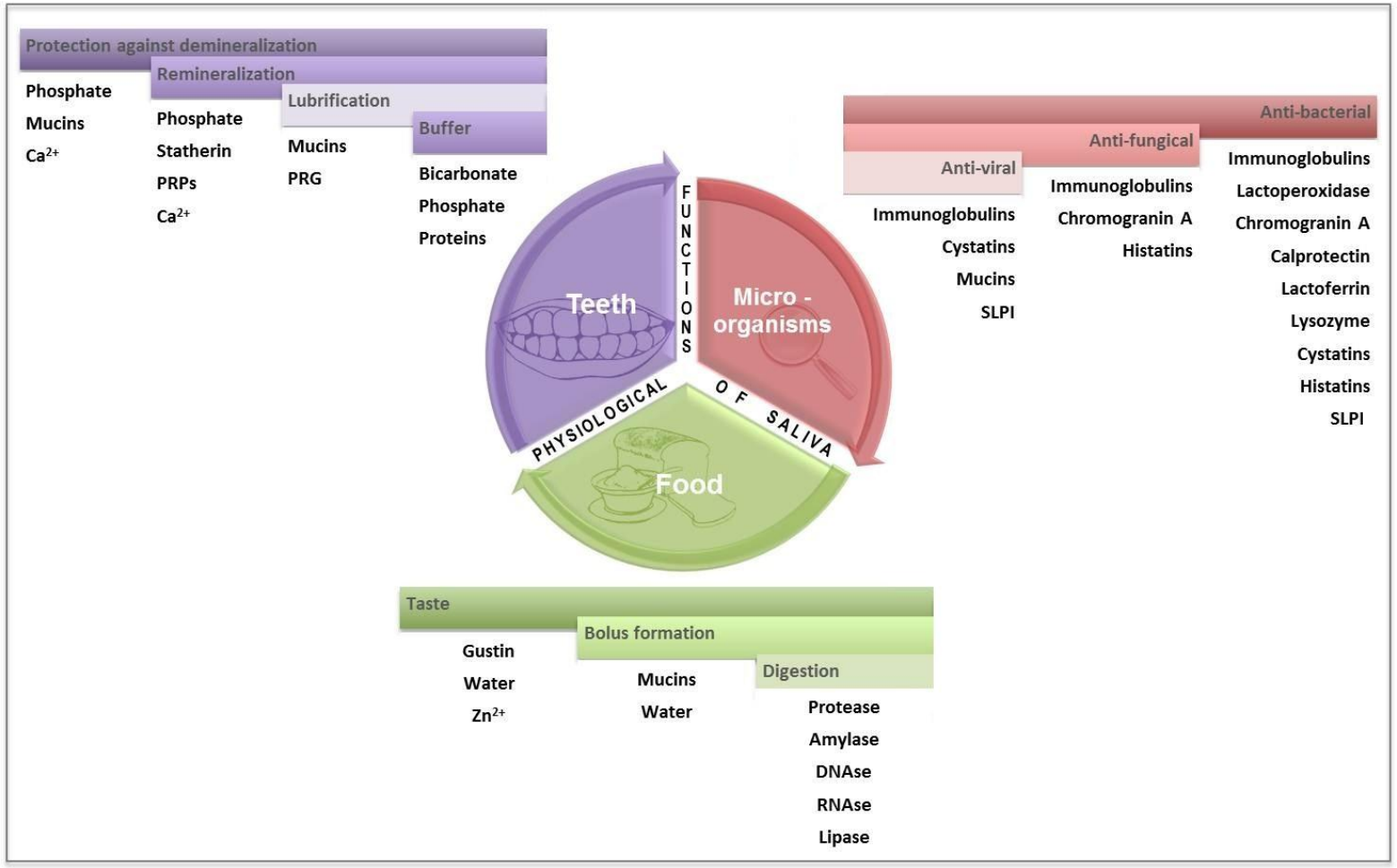

Figure 1: Illustration of major salivary protein constituents and their functional role in oral cavity health.

Human salivary glands are present under the form of a branched structure, with terminal secretory structures (acini) and ducts. Small ducts conduct saliva from the acini and converge to progressively larger ducts until reach the oral cavity (Ellis 1991; Moore *et al.* 2010). The main types of salivary gland acinar cells are mucous and serous. The distribution of these cells differs depending on the salivary gland (Figure 2). Acinar cells comprise about 90% of the gland and synthesize and secrete the majority of salivary proteins (Ellis 1991).

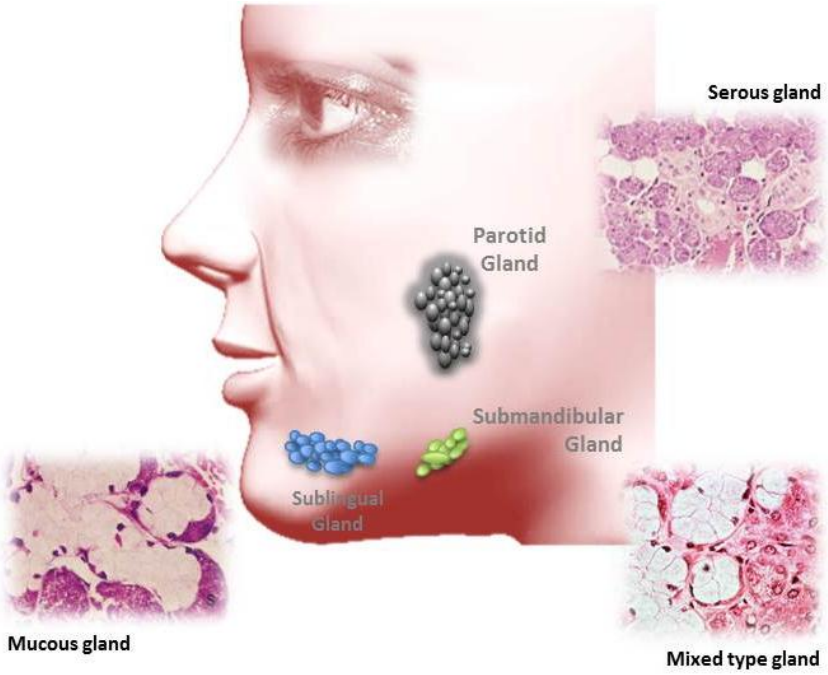

Figure 2: Schematic representation of human salivary glands anatomy and acinar structure.

Parotid glands are the largest salivary glands, located close to the external auditory pavilion, more precisely between the jaw branch and the mastoid. Parotid is a serous gland, composed of spherical shape serous acini, formed by serous cells. (Ellis 1991; Moore *et al.* 2010). Submandibular glands present a U shape and are located under the mandible. This gland is a mixed type gland, presenting both acini composed by serous cells (80%) and mucous cells (20%) (Ellis 1991; Moore *et al.* 2010).The sublingual glands are located at the floor of the mouth and consist mostly of mucous acini capped with serous demilunes, being therefore categorized as a mucous gland. (Ellis 1991; Moore *et al.* 2010). There are still about 500 to 1000 minor salivary glands located throughout the oral cavity, distributed by the lip and mouth mucosa, tongue and palate (Ellis 1991; Moore *et al.* 2010). Acinar cells secrete more than 85% of saliva proteins, not being negligible the

contribution of glandular duct cells, responsible for secretion of proteins with important biological functions such as growth factors, immunoglobulins and kallikreins (Vitorino *et al.* 2004; Amado *et al.* 2005; Esser *et al.* 2008; Castagnola *et al.* 2011).

Besides salivary gland secretions, other sources contributes to whole saliva proteome namely serum filtrate, gingival crevicular fluid, bacteria and bacterial products, viruses, fungi, desquamated cells from oral epithelium and food debris (Vitorino *et al.* 2004; Amado *et al.* 2005; Dodds *et al.* 2005; Esser *et al.* 2008). A distinctive characteristic of saliva in comparison with traditional biofluids is the fact that 20-30% of all identified peptides belong to the main salivary peptide classes, namely statherin, PRPs, histatins and SMR3B (Amado *et al.* 2012).

Despite the unknown biological function of several salivary proteins, the role of PRPs is clearly associated to oral health. Lactoferrin, in conjunction with peroxidase, is a potent inhibitor of cariogenic bacteria system (Aps *et al.* 2005). Statherin, another characteristic salivary protein, allows saliva to keep their state oversaturated of calcium and phosphate salts, contributing to the maintenance of an intact dentition and inhibiting spontaneous precipitation of calcium phosphate. The salivary histidine-rich proteins, histatins, are small proteins with anti-fungal activity (Vitorino *et al.* 2004; Dodds *et al.* 2005; Vitorino *et al.* 2006) (Figure 1). Saliva is also composed by high molecular weight glycoproteins, the mucins, which are important organic components of this fluid. Mucins present a high degree of glycosylation and hydration potential, able to prevent dehydration and provide the necessary lubrication of oral cavity. These glycoproteins are either involved in toxins binding and bacteria agglutination, being important components of the acquired pellicle (Dodds *et al.* 2005; Vitorino *et al.* 2007) (Figure 1). Although bacteria are commonly referred as part of saliva, bacterial proteins identification in saliva is limited, being only possible when multidimensional approaches are used in saliva (Vitorino *et al.* 2012).

1.3.2. Proteome profiling of saliva

In the last decade, the combination of advanced MS-based technologies with the development of bioinformatics tools, enabled an important progress in disclosing the complete salivary proteome and peptidome using proteomics (Amado *et al.* 2012). These advances enhanced saliva potential as a diagnostic fluid not only for oral pathologies, but also for systemic diseases (Samaranayake 2007; Castagnola *et al.* 2011; Malamud 2011).

There are several proteomics approaches applied to the study of biological fluids, but the proteome cannot be resolved completely using a single proteomic technology. The

analysis of high complexity samples as saliva requires previous fractionation steps and the combination of multiples techniques to analyze and cover a large spectrum of the proteome (Figure 3). The reduction of sample complexity is necessary because the high abundant proteins can mask the detection of the lower abundant ones (Rodriguez-Suarez *et al.* 2012). In protein analysis the most common methods used are one- and two-dimensional gel electrophoresis (1-DE and 2-DE), one- and two-dimensional liquid chromatography (1D-LC and 2D-LC), coupled with MS (Guo *et al.* 2007; Matt *et al.* 2008; Issaq *et al.* 2009). Hyphenated techniques, 2-DE-MS and HPLC-MS, allow the identification of a large number of proteins in complex mixtures, becoming the most used tools in proteomics (Watso *et al.* 2007).

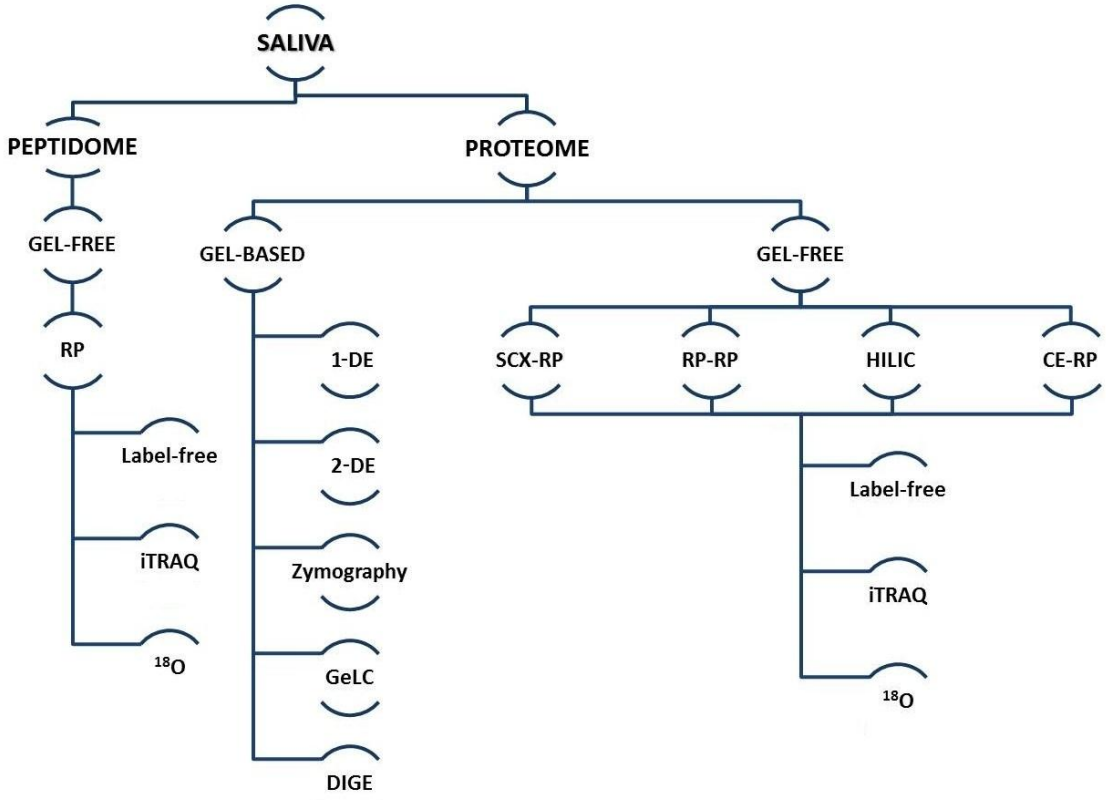

Figure 3: Flowchart of the common strategies used for saliva proteome/peptidome characterization.

The 2-DE, one of the first tools used in proteomics, introduced by O'Farrell and Klose (1975), remains an useful and actual platform for the separation of complex mixtures of proteins in greater number of fractions than traditional 1-DE. In the first dimension, the isoelectric focusing (IEF), the separation of proteins is based on their native charge, while in the second dimension, SDS-PAGE, are separated by molecular weight (MW). The

result is a set of *spots* to which can be assigned specific *X* and *Y* coordinates, unlike protein bands obtained in one-dimensional techniques. Each *spot* of the gel contains one or a very small number of proteins, depending on the complexity of the sample. Thus, thousands of proteins can be separated in a single gel, allowing the determination of the p*I* values, MW and relative abundance. Another aspect of 2-DE is the ability to separate proteins based on the presence of post-translational modifications (PTMs) (Klose 1975; O'Farrell 1975; Issaq *et al.* 2009). This technique has been applied to saliva, being generally employed for biomarker discovery, namely of oral (dental caries, gingivitis and periodontitis) (Vitorino *et al.* 2006; Hu *et al.* 2008; Wu *et al.* 2009; Goncalves *et al.* 2011) and systemic diseases (Sjögren syndrome, Rheumatoid arthritis, oral squamous cell carcinoma and DM) (Ferrary *et al.* 2002; Hirtz *et al.* 2006; Giusti *et al.* 2010; Baldini *et al.* 2011) as well as to evaluate specific conditions like smoking (Jessie *et al.* 2010) or orosensory stimulation (Lorenz *et al.* 2011) effects on saliva proteome. As main disadvantage, this technique present limitations on its applicability to high or very low molecular weigh separated proteins, to hydrophobic membrane proteins and to low abundance proteins that fail to be detected. (Matt *et al.* 2008; Rodriguez-Suarez *et al.* 2012). Accordingly to Bland *et al.* (2010) to obtain reliable and confident data with 2-DE several replicates per sample are required to achieve a coefficient variation ranging between 20–30%. More recently, an improvement in 2-DE, applying fluorescent labels to samples, named two dimensional difference gel electrophoresis (2D-DIGE), allowing the analysis of two or three marked protein samples in the same gel, reducing gel-to-gel variation and increasing reproducibility (Unlu *et al.* 1997; Tonge *et al.* 2001; Beckett 2012). This strategy enabled the evaluation of salivary protein profiles among head and neck squamous cell (Dowling *et al.* 2008), breast (Streckfus *et al.* 2012) and lung (Xiao *et al.* 2012) cancer patients.

In the last decade, other methods for separating proteins have been exploited in proteomics and most of the quantitative proteomic studies moved from 2-DE technology to gel-free approaches (Neverova *et al.* 2005; Rodriguez-Suarez *et al.* 2012). Many involve liquid chromatography technologies (1D-LC and 2D-LC) that use solid- and liquid-phase media to separate protein or their peptides fragments (Guo *et al.* 2007; Matt *et al.* 2008). 1D-LC can be used to separate proteins according to their molecular mass, isoelectric point or hydrophobicity. In 2D-LC, proteins are separated in the first dimension by isoelectric point and in second dimension by hydrophobicity, thereby increasing the extent of protein fractionation compared with 1D-LC (Guo *et al.* 2007; Matt *et al.* 2008). Liquid Chromatography (LC) technologies help to reduce sample complexity, being a common

strategy the use of a preparative chromatography previous to LC-MS/MS analysis (Rodriguez-Suarez *et al.* 2012). The use of peptide strong cation exchange (SCX), 2D reversed phase–reversed phase (RP-RP) chromatography or hydrophilic interaction chromatography (HILIC) enables the sample fractionation and increase substantially proteome coverage (Rodriguez-Suarez *et al.* 2012). The combination of successive chromatographic steps has been termed multidimensional protein identification technology (MudPIT) and increase the dynamic range of protein identification (Chiou *et al.* 2011).

The most common method of MS-based proteomics is conducted by the digestion of protein mixture in short peptides (Zhou *et al.* 2008). The analysis of the resulting fragments by MS leads to protein identification and allows partial sequencing of target protein, as well as evaluates the presence of PTMs (Tanaka *et al.* 2009). This approach, referred as bottom-up, includes three strategies for protein identification: peptide mass fingerprinting (PMF), peptide fragment fingerprinting (PFF) and *de novo* sequencing (Tanaka *et al.* 2009). In the case of PMF, the *m/z* ratio obtained for each peptide after enzymatic or chemical digestion is accurately measured and compared with all theoretical masses present in databases obtained by *in silico* proteolytic digestion (Henzel *et al.* 2003). The confidence level in protein identification is extremely dependent on the correlation between the mass detected and the theoretical mass (Dakna *et al.* 2009). In the identification by PFF precursor's peptides are selected and subject to tandem mass spectrometry (MS/MS). The most commonly used activation method in MS/MS of peptides is energetic collisions with a neutral target gas, commonly referred as collision-induced dissociation (CID) (Zhou *et al.* 2008). According to the peptide fragmentation model, the resulting fragments are compared with the theoretical masses obtained *in silico* (Amado *et al.* 2005; Watso *et al.* 2007). The progress observed in protein identification and characterization is close related with mass spectrometers evolution (Chen 2008; Xia *et al.* 2008; Amoresano *et al.* 2009; Gelpi 2009). The analysis of proteins/peptides by MS produces a considerable amount of data, being necessary the use of bioinformatics (Canas *et al.* 2006). There are several programs able to perform the analysis, of which are examples SEQUEST (Sadygov *et al.* 2004) and MASCOT (Perkins *et al.* 1999). These programs are powerful search engines that correlate uninterpreted MS spectra of peptides with amino acid sequences from protein and nucleotide databases to identify proteins. (Canas *et al.* 2006; Dakna *et al.* 2009).

Recently, the development of mass spectrometric methods has not only the aim of protein identification, but also the quantification, enabling the comparison of the relative levels of protein expression between two or more different samples (Huzarewich *et al.* 2010; Xie *et*

al. 2011). Application of LC-MS-based quantitative proteomics has become increasingly applied due to capabilities for proteome coverage and methods precision and accuracy (Quintana *et al.* 2009; Wei *et al.* 2009; Westman-Brinkmalm *et al.* 2009; Huzarewich *et al.* 2010; Chiou *et al.* 2011; Xie *et al.* 2011; Rodriguez-Suarez *et al.* 2012). The most common strategy for relative and absolute quantification, known as bottom-up or shotgun proteomics, relies on protein digestion followed by a fractionation, a critical process to avoid the identification of only a portion of the proteins present, the higher abundant (McCormack *et al.* 1997; Becker *et al.* 2011; Rodriguez-Suarez *et al.* 2012). The strategy of labeling peptides with isotopes enables this methodology for differential expression, comparing samples by labeling them separately with molecular tags containing light or heavy isotopes (Huzarewich *et al.* 2010; Becker *et al.* 2011). The method known as Isotope Coded Affinity Tags (ICAT) relies on thiol specific reaction and provide quantitative information based on the ratios between heavy and light peptides (Chen 2008; Washburn 2011). Another approach relies in the incorporation of the heavy or light oxygen isotopes during proteolysis, referred as $^{16}O/^{18}O$ labeling, which allows quantitative comparison between two samples (Yao *et al.* 2001). This approach has been refined by Ross *et al.* (2004) with the development of Isobaric Tagging for Relative and Absolute Protein Quantification (iTRAQ) (Ross *et al.* 2004; Becker *et al.* 2011). iTRAQ method enables simultaneous determination of both the identity and relative abundances of peptide pairs using a peptide-fragmentation-based data analysis (Ross *et al.* 2004; Rodriguez-Suarez *et al.* 2012). This methodology provides a multiplex assay that is ideally suited for relative quantification and biomarker applications (Huzarewich *et al.* 2010; Rodriguez-Suarez *et al.* 2012) and a 8-plex kit has been commercialized by AB SCIEX Instruments (Rodriguez-Suarez *et al.* 2012). The strategy of isotopic labeling expanded with the application of different isotope-labeled reactants (Julka *et al.* 2004). Another method, the stable isotope labeling by amino acids in cell culture (SILAC), involves the in vivo metabolic labeling of a cell line or a small organism with isotope-labeled amino acids (Ong *et al.* 2002). This method present limitations related with the metabolic nature of the labeling, fact that pave the way to the development of a novel strategy for biomarker discovery, the Stable Isotope Labeled Proteome (SILAP) method (Shah *et al.* 2009; Yu *et al.* 2009). As an alternative to isotope labeling methods for protein quantification were developed label free methods, based on comparison of the normalized intensities of MS signal (Wang *et al.* 2003; Wiener *et al.* 2004) or on the frequency of identifications (Bondarenko *et al.* 2002; Liu *et al.* 2004). Label free LC-MS-based strategies also enable relative quantification of peptides by direct evaluation of peaks intensity and area

(Bondarenko *et al.* 2002). After an initial resistance, the validity of label free methods has been demonstrated and they become widely accepted (Becker *et al.* 2011). However, the experimental error resultant from run to run variations in LC and ion suppression should be taken in account (Rodriguez-Suarez *et al.* 2012).

1.3.3. Salivary proteomics and peptidomics in clinical diagnosis

Taking advantage of the methodologies referred above, a complete catalogue of the salivary proteome has been created, including its classification according to their parotid or submandibular/sublingual origins (Spielmann *et al.* 2011). Qualitative and quantitative proteomic approaches that contribute to a high-throughput analysis of saliva proteome and peptidome are relevant to harness the potential of saliva diagnostic and prognostic value (Granger *et al.* 2007; Hu *et al.* 2007; Castagnola *et al.* 2011; Pfaffe *et al.* 2011; Brinkmann *et al.* 2012). According to Spielmann *et al.* (2011) the systematic study of all salivary secretory proteome components, PTMs, protein complexes and its classification is an initial key step for saliva to be used for clinical applications. Information related to the effect of diurnal variation on saliva composition (Hardt *et al.* 2005) as well as the evaluation of glandular secretions contribution (Bandhakavi *et al.* 2011) were assessed using stable isotope labeling quantitative approaches. So far, more than 3000 different proteins (Hu *et al.* 2005; Xie *et al.* 2005; Denny *et al.* 2008; Bandhakavi *et al.* 2009; Yan *et al.* 2009) and 2000 peptide species were identified (Huq *et al.* 2007; Schipper *et al.* 2007; Helmerhorst *et al.* 2008; Huang *et al.* 2009; Lucchi *et al.* 2009; Vitorino *et al.* 2009; Amado *et al.* 2010; Morzel *et al.* 2012).

Several studies aiming biomarker discovery in saliva have been performed in the last years, highlighting the growing importance of salivary proteomics and peptidomics in clinical diagnosis (Spielmann *et al.* 2011; Al Kawas *et al.* 2012; Amado *et al.* 2012; Liu *et al.* 2012). Saliva proteome analysis in several pathological conditions such as bleeding oral cavity, caries, oral lichen planus, periodontitis, Sjögren's syndrome, systemic sclerosis, graft versus host disease, oral cavity cancer, head and neck cancer (HNC), breast cancer, gastric cancer, type 1 DM and type 2 DM was reviewed by Al-Tarawneh *et al.* (2011) and 180 differentially expressed proteins were identified.

In an initial phase, salivary diagnostic approaches have been developed to monitor oral diseases such as caries risk, gingival and periodontal diseases (Al-Tarawneh *et al.* 2011; Spielmann *et al.* 2011; Amado *et al.* 2012). Vitorino *et al.* (2006) applied a proteomic approach to evaluate the influence of salivary protein composition on *in vitro* dental pellicle formation and its possible correlation with dental caries. The study involved the

comparison of caries free and caries susceptible subject's, being observed differences in the levels of acidic PRPs, lipocalin and cystatins (S and SN). The higher levels of cystatins, physiological inhibitors of cathepsins, found in caries-free subjects suggest that inhibition of proteolytic events on other salivary proteins may indirectly provide tooth protection as well as the higher levels of the phosphorylated acidic PRPs 1/2 in caries-free subjects. Preza *et al.* (2009) conducted a study in parotid saliva aiming to evaluate if glandular functional status could be associated with the presence of root caries in elderly and identified 14 biomarkers. Despite of the inter-individual variations in protein patterns, parotid function tends to change upon aging, with implications for dental caries activity.

Proteomic analyses become an important tool to discover potential markers of gingivitis and/or periodontitis like alpha-2-macroglobulin, alpha-1-antitripsin, elastase and albumin (Fabian *et al.* 2007; Fabian *et al.* 2008). Other proteins levels like immunoglobulin, molecular chaperone Hsp70, cystatin S, salivary amylase, calprotectin, histatins, lysozyme, lactoferrine, defensins, peroxidases, PRPs and mucins could present a predictive value for the evolution of gingivitis to periodontitis (Fabian *et al.* 2008). Gonçalves *et al.* (2011) compared the proteomic profile of whole saliva from gingivitis patients and healthy controls and observed that gingival inflammation was associated with increased levels of albumin, hemoglobin, immunoglobulin peptides and keratins. Salivary cystatin levels appeared to be more abundant in healthy subjects. A study conducted by Ito *et al.* (2008) evaluated the amounts of antimicrobial proteins (cystatins and lysozyme) in periodontitis. Saliva from patients with periodontal disease presented lower levels of cystatin and lysozyme in comparison to the healthy group, being suggested as potential biomarkers for periodontitis development. Wu *et al.* (2009) compared the proteomic profile of whole unstimulated saliva of subjects with aggressive periodontitis versus control group. The levels of serum albumin, immunoglobulin gamma2 chain C region, immunoglobulin alpha2 chain C region, vitamin D-binding protein, salivary alpha-amylase and zinc-alpha2 glycoprotein were increased in patients, whereas lactotransferrin, elongation factor 2, 14-3-3 sigma, short palate, lung and nasal epithelium carcinoma-associated protein 2 precursor and carbonic anhydrase VI were decreased.

Sjögren's syndrome (SS) is a systemic autoimmune disease, manifested by severe impairment of exocrine gland function and focal mononuclear cell infiltrates within the lacrimal and salivary glands (Delaleu *et al.* 2005). Baldini *et al.* (2007) evaluated the salivary composition of SS patients in comparison to a control group and observed a different protein pattern characterized by a remarkable decrease in carbonic anhydrase VI

levels as well as set of differentially expressed proteins related to acute and chronic inflammation and/or involved in oxidative stress injury.

A proteomic approach conducted by Peluso *et al.* (2007) evaluated modifications of salivary peptides in patients with SS before and after pilocarpine treatment. The administration of this parasympathomimetic drug in adult patients restored the levels of several salivary proteins. The authors also reported an increased level of alpha-defensin 1 and beta-defensin 2 in patients, suggesting them as potential markers of oral inflammation in SS patients. Fleissig *et al.* (2009) studied the salivary protein profile of human unstimulated whole saliva and showed protein expression differences in SS patients compared to healthy subjects. Baldini *et al.* (2011) conducted a study aiming to refine the diagnostic power of a panel of potential salivary biomarkers described in primary SS with respect to both healthy volunteers and pathological controls. The results showed 15 differently expressed proteins, namely alpha-amylases precursor, carbonic anhydrase VI, β-2 microglobulin, GAPDH, epidermal fatty acid binding protein and immunoglobulin k light chain between healthy subjects and non-SS pathological controls. The authors concluded that saliva might represent a novel milieu for the discovery of candidate biomarkers for primary SS diagnosis, and deepen knowledge on the pathophysiology underlying glandular and systemic autoimmune disorders.

Research on salivary biomarkers revealed potential tools for oral cavity cancer diagnosis (Lima *et al.* 2010) as well as for the detection of malignant tumors that are remote from the oral cavity (Bigler *et al.* 2009). In respect to oral cancer detection, Hu *et al.* (2008) discovered and validated a discriminatory panel comprising mac-2 binding protein, myeloid-related protein 14, CD59 and catalase with a clinical accuracy greater than 90%. In oral squamous cell carcinoma patients saliva was also detected a higher concentration of interleukin (IL)-8 (St John *et al.* 2004). De Jong *et al.* (2010) used advanced MS-based quantitative approach for saliva proteome profiling in oral cancer and proposed salivary actin and myosin abundances to distinguish oral lesion types. Contucci *et al.* (2005) reported a sensible reduction of the statherin level in the saliva of patients with precancerous and cancerous lesions of the oral cavity compared with healthy subjects. The results of Pickering *et al.* (2007) demonstrate the potential utility of salivary endothelin-1 levels to monitor patients at risk for oral squamous cell carcinoma.

According to Li *et al.* (2004) the analysis of HNC patients' saliva enables the diagnosis with 91% precision, with great importance in early diagnosis and treatment success. The detection of HNC in all stages is possible evaluating salivary levels of CD44 protein

(Franzmann *et al.* 2005). Ohshiro *et al.* (2007) observed a differentially protein expression between HNC patients and healthy subjects saliva. The authors reported the absence of common salivary cystatin S, parotid secretory factor and poly-4-hydrolase beta-subunit proteins and the presence of alpha-1-B-glycoprotein and complement factor B proteins in patients affected by HNC. Dowling *et al.* (2008) reported significantly increased levels of beta fibrin (2.77-fold), S100 calcium binding protein (5.35-fold), transferrin (3.37-fold), immunoglobulin heavy chain constant region gamma (3.28 fold) and cofilin-1 (6.42 fold) in HNC patients saliva. The authors also observed a significant decrease of transthyretin (2.92 fold) in patients in comparison with controls.

Saliva has already been analyzed with the purpose of breast cancer diagnosis (Streckfus *et al.* 2008; Streckfus *et al.* 2012). Streckfus *et al.* (2008) identified 49 proteins that discriminate healthy patients from those with breast cancer. Salivary protein analysis also enables the differentiation of breast tumors malignity (Lima *et al.* 2010). In another approach, Wu *et al.* (2009) proposed a screening method for gastric cancer in saliva, based in the significant differences observed in the mass to charge ratio (m/z) peaks of proteins with 1472.78 Da, 2936.49 Da, 6556.81 Da and 7081.17 Da, between patients and healthy subjects.

A critical analysis of differentially expressed salivary proteins among distinct pathologies evidenced, for instance, that cystatin SA-III was upregulated in pathologies like bleeding oral cavity, SS and breast cancer, being a non-specific disease biomarker (Al-Tarawneh *et al.* 2011). The downregulated expression of acidic PRP-1 was observed in SS and type 1 DM. The expression of alpha-amylase seems disease dependent, being upregulated in SS, dental caries and periodontitis and downregulated in type 1 DM (Al-Tarawneh *et al.* 2011). Amado *et al.* (2012) evaluated the differentially expressed salivary proteins in the presence of pathophysiological conditions and performed an interaction analysis using Cytoscape for network visualization. Clusters were generated based on disease-modulated protein levels and the methodology used for protein separation and identification, and no clear association between a specific protein and a disease was observed. The authors reported that several proteins like cystatin S, cystatin C, amylase, defensins and statherin seem to be similarly modulated by diseases like dental caries, periodontitis, SS, HNC and diabetes.

The systematic analysis of salivary peptidome might provide new insights in clinical diagnosis and in the characterization of some pathophysiological conditions. The

proteolytic fragmentation pattern could also contribute to this objective, being seen as a "fingerprint" of the physiological status (Amado *et al.* 2012).

Several saliva peptidome profiling approaches aiming unraveling novel potential biomarkers for pathologies like autism, caries and oral diseases, HNC and diabetes have been developed (Castagnola *et al.* 2008; Huang *et al.* 2009; Cabras *et al.* 2010; Vitorino *et al.* 2010; Zhang *et al.* 2012). Castagnola *et al.* (2008) performed a comparative analysis to evaluate salivary phospopeptidome in autistic patients. In particular, the phosphorylation level of statherin, histatin 1 and acid PRPs was significantly lower in a sub-group of autistic patients. The authors argue that these results provide a clue regarding some potential molecular events at the basis of the disease, namely the relation of hypo-phosphorylation of salivary peptides to possible asynchronies in the phosphorylation of proteins involved in the development of central nervous system. Rudney *et al.* (2009) performed a comparative study of patients grouped accordingly to the clinical outcome evaluated by the levels of caries, supragingival plaque, total streptococci and *Tannerella forsythensis*. Statherin and truncated cystatin S levels showed significant inverse correlations with caries and all the microbiological variables and may be potential risk indicators for the development of caries and other oral diseases. Inzitari *et al.* (2006) detected various fragments and derivatives of statherin and P-B peptide in human saliva and suggested that these salivary peptides are subjected to post-translational proteolytic cleavages. This discovery could be related with physiological processes of oral and dental microenviroment and might be relevant for oral cavity defense from different pathogens and for the modulation of oral flora growth. Amado *et al.* (2012) evaluated salivary peptidome of 10 patients with HNC and identified 1,834 fragments belonging to 289 unique proteins. From these, 158 were only identified in HNC patients, mostly involved in gene expression regulation, extracellular matrix organization and tissue development.

1.4. Aplication of salivary proteomics and peptidomics to DM

Although there is no consensus about the changes in the composition of saliva induced by DM, an increased concentration of glucose, peroxidase, potassium, calcium, total protein, amylase, immunoglobulin, lactoferrin, myeloperoxidase, albumin and advanced glycation end products (AGEs) has been described (Ben-Aryeh *et al.* 1988; Sreebny *et al.* 1992; Ben-Aryeh *et al.* 1993; Yavuzyilmaz *et al.* 1996; Yoon *et al.* 2004). A decrease in salivary flow, (related or not with symptoms of xerostomia), content in sialic acid, pH, levels of magnesium and zinc was also reported (Banoczy *et al.* 1987; Ben-Aryeh *et al.* 1988; Anderson *et al.* 1993; Yavuzyilmaz *et al.* 1996; Belce *et al.* 2000; Mata *et al.* 2004; Caldeira *et al.* 2005). These biochemical changes in saliva of diabetics are not only related to a dysfunction of the salivary glands secretion, but also with structural and morphological modifications (Lotti *et al.* 1988; Caldeira *et al.* 2005). These changes in the inorganic and organic composition of salivary secretions may explain the higher susceptibility to oral infections, as well as dental and periodontal diseases accompanied by problems in healing. Indeed, it has been described in DM patients a higher prevalence and severity of periodontal disease. A frequent complaint among diabetics is xerostomia, which is strongly associated with a reduced salivary flow and other oral symptoms (Sreebny *et al.* 1992; Ben-Aryeh *et al.* 1993).

Qualitative and quantitative proteomic approaches have been developed to better characterize DM-modulated salivary proteome and peptidome, particularly in type 2 DM (Rao *et al.* 2009; Border *et al.* 2012; Chan *et al.* 2012). Rao *et al.* (2009) using 2D-LC-MS/MS identified 487 proteins in saliva of type 2 DM patients, of which 65 proteins were increased greater than 2-fold in patients in comparison to healthy subjects. Altered expressed proteins were associated with metabolic and immune pathways, and from these alpha-2 macroglobulin, alpha-1-antitrypsin, cystatin C and transthyretin were upregulated in DM. The authors further validated the proteomic approach assessing the levels of alpha-2 macroglobulin, alpha-1-antitrypsin, cystatin C, transthyretin and salivary alpha-amylase by western blotting and ELISA (Rao *et al.* 2009).

Chan *et al.* (2012) conducted a study involving the comparison of saliva 2-DE profiles of type 2 DM subjects with periodontitis and with healthy periodontium. The comparative analysis showed 7 proteins with significant differential expression. Actin-related protein, carbonic anhydrase VI, IL-1 receptor antagonist and polymeric immunoglobulin receptor were downregulated while immunoglobulin J chain, leukocyte elastase inhibitor and plastin-2 were upregulated in saliva of diabetic patients with periodontitis. The authors

concluded that these proteins are involved in the physiological response towards periodontitis with potential application as biomarkers for the prediction of type 2 diabetic patients in risk of periodontitis development.

Using label-free quantitative approach, Border *et al.* (2012) explored the proteome profile of saliva and found 96 peptides corresponding to 52 proteins differentially expressed between type 2 DM diabetic edentulous patients and controls. Some diabetes-related inflammatory biomarkers including GAPDH and serum amyloid A were increased in diabetic subjects in opposition to decreased levels of amylase, palate, lung and nasal epithelium associated protein (PLUNC) and serotransferrin. In contrast with previous works (Rao *et al.* 2009; Chan *et al.* 2012), salivary carbonic anhydrase VI, alpha-2-macroglobulin, PLUNC and uteroglobin were identified in lower levels in diabetic patients (Border *et al.* 2012). The authors argue that despite this exploratory study further validation in a larger population is required to evaluate the potential use of these differentially expressed proteins as diabetes biomarkers (Border *et al.* 2012).

Regarding the evaluation of type 1 DM salivary proteome and peptidome, very few studies are known. Hirtz *et al.* (2006) used 2-DE-MALDI-TOF/MS to compare the salivary proteomes of poorly controlled type 1 diabetic patients and controls and identified 23 proteins modulated by the disease. Most of those proteins were underexpressed and corresponded to isoforms of alpha-amylase (14), isoforms of acidic PRPs (3), isoforms of salivary cystatin SA-1 (3) and to prolactin inducible protein. Two isoforms of serotransferrin, known to be implicated in the oral anti-inflammatory process were overexpressed in patients, suggesting that this pathological condition induces a decrease of non-immunological defense of oral cavity. Cabras *et al.* (2010) analyzed the composition of the acid soluble fraction of whole saliva by RP-HPLC-ESI-MS in children and adolescents with DM and compared to sex- and age-matched control subjects. The results showed significant lower levels of statherin, SMR3B and histatins, while the concentration of alpha-defensins 1, 2 and 4, S100A9 and several small peptides were increased. This pioneer study highlighted the impairment of salivary peptides involved in the safeguard of the oral cavity in children with type 1 DM that may contribute to the major incidence of dental and periodontal diseases in these patients. The low concentration of P-C (aPRP103-169) peptide was accompanied by high levels of some of its fragments, fact that could be explained by an increased salivary proteolytic activity.

In overall, the potential of saliva for biomarker discovery to DM, namely type 1, and related complications is quite unexplored being proteomic and peptidomics approaches

promisor’s tools to achieve this goal. Such efforts will promote a deeper knowledge about saliva proteome/peptidome adaptability and the ongoing development of salivary-based diagnosis with clinical advantageous outcomes.

CHAPTER I

AIMS

AIMS

Taking into account the growing interest in saliva as diagnosis fluid namely for type 1 DM, the general goal of the present thesis was to characterize type 1 DM-induced alterations in salivary proteome and peptidome, with the perspective of improving understanding of the pathophysiology of the disease and unraveling novel potential biomarkers, mainly for DM-related microvascular complications. To accomplish this, specific purposes were outlined in the original research articles (Studies I, II and III) that comprise chapter II:

i) to characterize type 1 diabetes-induced alterations in the salivary peptidome aiming to find prospective biomarkers for type 1 DM oral health evaluation and to investigate the extent and mode of salivary proteolysis, as well as identify the potential proteases responsible for the oral health alterations in patients (Study I).

ii) to disclose the proteolytic events underlying type 1 DM and related microvascular complications by performing a straightforward screening of the proteases presents in the bodily fluids saliva, serum and urine (Study II).

iii) to evaluate the effect of long-term type 1 DM and related microvascular complications on salivary proteome and peptidome with iTRAQ-based quantitative approach, aiming to identify potential protein and peptide targets for disease diagnosis (Study III).

List of original studies:

Study I - Caseiro A, Vitorino R, Barros AS, Ferreira R, Calheiros-Lobo MJ, Carvalho D, et al. Salivary peptidome in type 1 diabetes mellitus. Biomedical Chromatography. 2012;26(5):571-82.

Study II - Caseiro A, Ferreira R, Quintaneiro C, Pereira A, Marinheiro R, Vitorino R, et al. Protease profiling of different biofluids in type 1 diabetes mellitus. Clinical Biochemistry. 2012;45(18):1613-9.

Study III - Caseiro A, Ferreira R, Quintaneiro C, Pereira A, Marinheiro R, Vitorino R, et al. Salivary proteome and peptidome profiling in type 1 diabetes mellitus using a quantitative approach (Under review in Journal of Proteome Research).

CHAPTER II -

EXPERIMENTAL WORK

STUDY I -

SALIVARY PEPTIDOME IN TYPE 1 DIABETES MELLITUS

Research article

Biomedical Chromatography

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Salivary peptidome in type 1 diabetes mellitus

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ABSTRACT: Diabetic patients show a high susceptibility to oral diseases of inflammatory, catabolic and chronic nature with potential impact on saliva composition. In this study, our purpose was to characterize type 1 diabetes-induced alterations in the salivary peptidome aiming to find prospective biomarkers for type 1 diabetes oral health evaluation. Peptidomic analysis of saliva from controls ($n = 5$) and type 1 diabetic patients ($n = 5$) were performed by liquid chromatography followed by mass spectrometry. The proteolytic activity and metalloproteinases expression was accessed by zymography and slot blot analysis, respectively. Data evidenced a significant increase in the percentage of peptides in diabetic patients paralleled by a higher proteolytic activity, compared with healthy individuals. The nonsalivary gland protein fragments identified in saliva were mainly derived from collagen and extracellular matrix proteins, namely collagen type I. The cleavage site frequency analysis showed significant differences between healthy and type 1 diabetic individuals, highlighting the activity of proteases such as matrix metalloproteinase-9 and cathepsin D. Our results highlight salivary collagen fragments as potential biomarkers to follow up diabetes-related oral damage. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information can be found in the online version of this article.

Keywords: saliva; proteomics; diabetes; proteolysis; mass spectrometry

Introduction

Saliva's use for diagnosis is gaining popularity in the fields of dentistry, physiology, internal medicine, endocrinology, pediatrics, immunology and forensic medicine, with it being used in the reliable assessment of an increasing number of drugs, hormones and antibodies (Dodds *et al.*, 2005; Lima *et al.*, 2010; Wu *et al.*, 2010; Yeh *et al.*, 2010). Saliva presents the advantage of being easily collected with noninvasive procedures, making it an attractive alternative to blood samples. Its use in diagnosis is even more attractive in children and the elderly, where collecting a blood sample has more risks (Lima *et al.*, 2010; Palanisamy and Wong, 2010; Yeh *et al.*, 2010). Moreover, analysis of saliva may provide a cost-effective approach in screening oral and systemic diseases in large populations (Lima *et al.*, 2010; Samaranayake, 2007; Yeh *et al.*, 2010).

Whole saliva contains specific proteins produced by the salivary glands and others derived from crevicular fluid, mucosal tissue, bacteria and bacterial products, viruses, fungi and cellular debris. The acinar cells of the salivary glands secrete more than 85% of salivary proteins, being the glandular duct cells responsible for the secretion of proteins with important biological functions such as growth factors, immunoglobulins and kallikreins (Esser *et al.*, 2008; Vitorino *et al.*, 2004, 2005). About 40–50% of the whole saliva proteome corresponds to small proteins and peptides (Amado *et al.*, 2010) further supported by oral cavity proteolysis (Vitorino *et al.*, 2009). This saliva's protein fraction, usually known as the salivary peptidome, varies in terms of quantity and quality owing to several factors such as time of day, individual physiology and health status (Amado *et al.*, 2010).

In fact, the proteolytic activity in the oral environment is associated with dental caries susceptibility and periodontal disease status (Vitorino *et al.*, 2006). Because of their greater susceptibility to bacterial infections, diabetic patients present a higher risk of these oral diseases (Lamster *et al.*, 2008; Negrato and Tarzia, 2010).

Type 1 diabetes mellitus accounts for about 5–10% of individuals with diabetes, mostly resulting from an autoimmune destruction of pancreatic islet β cells (Alberti and Zimmet, 1998). An impairment of polymorphonuclear neutrophils function with abnormalities of adherence, chemotaxis and phagocytosis have been well described (Manouchehr-Pour *et al.*, 1981; Ryan *et al.*, 2003) and correlated to the severity of periodontal disease (Manouchehr-Pour *et al.*, 1981). Uncontrolled type 1 diabetes mellitus is associated with inflammation, as evidenced by

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Abbreviations used: ACN, acetonitrile; FA, formic acid; MMPs, matrix metalloproteinases; NSGP, nonsalivary gland peptides; PLS-DA, partial least squares – discriminant analysis; SGP, salivary gland secreted peptides; T1D, type 1 diabetic; TBS, Tris buffered saline; TFA, trifluoroacetic acid; TBS-T, TBS-Tween.

increased inflammation markers like C-reactive protein, adhesion molecules and interleukins (Devaraj *et al.*, 2006; Jialal *et al.*, 2007). As the disease progresses, lymphocytes and mononuclear cells infiltrate an increasing percentage of gingival tissue (Havemose-Poulsen and Holmstrup, 1997). Monocytes and macrophages produce interleukin-1, which affects fibroblast response with respect to cell proliferation, collagen and matrix metalloproteinases (MMPs) synthesis (Havemose-Poulsen and Holmstrup, 1997). Several studies have reported an increase of collagenase activity in gingival crevicular fluid of type 1 diabetic (T1D) patients, mainly originating from neutrophils and in specific circumstances also from fibroblasts and chondroblasts (Salo *et al.*, 1995; Sorsa *et al.*, 1992). Alterations in the activity rate of these proteases, specifically in dental plaque, have been related to periodontal disease status (Helmerhorst, 2007; Vitorino *et al.*, 2006, 2009).

The observation of a consistent salivary protein degradation pattern in subjects with similar oral health status can pave the way for exploiting this feature for diagnosis purposes (Helmerhorst, 2007). In this sense, the aim of our work was to characterize T1D salivary peptidome and establish the major protein cleavage sites, in order to investigate the extent and mode of salivary proteolysis, as well as identify potential proteases responsible for the oral health alterations in these patients.

Experimental

Reagents

HPLC-grade acetonitrile (ACN; Riedel, Seelze, Germany) and Milli-Q grade water were used. General chemical reagents such as ammonium bicarbonate, gelatin, Tris–HCl, $CaCl_2$, $ZnCl_2$, trifluoroacetic acid (TFA), protease inhibitor cocktail, formic acid (FA), CHCA, glycerol, Triton X-100 and Tween 20 were purchased from Sigma (Karlsruhe, Germany). Sequencing-grade modified trypsin (porcine) was from Promega (Madison, WI, USA). Mouse monoclonal antibodies anti-MMP-1 (clone 36665), MMP-2 (clone 101721) and MMP-9 (clone 36020) were acquired from R&D Systems (Minneapolis, USA).

Whole-saliva collection

Unstimulated saliva was collected from five healthy male subjects (aged 18–29 years) and five well-controlled T1D patients (four males and one female; aged 14–33), followed-up by the endocrinology service of Hospital São João – Porto. This study was approved by the local ethics committee and all participating individuals gave their written informed consent. At the moment of saliva collection a specialized dentist examined all participating subjects using as exclusion criteria the presence of gingivitis or other inflammatory symptoms for healthy individuals. Through the clinical observation, all the T1D patients presented gingivitis. The level of blood glycated hemoglobin (HbA1c) was accessed and presented normal values in controls (below 5.9%) and ranged from 6.7 to 8.4% in T1D patients. Unstimulated whole saliva was collected from all subjects, who had refrained from eating and drinking for at least 2 h (performed between 9:00 and 12:00 a.m.), by direct draining into an ice-cold saliva collection tube. The sample collection time was registered for each individual. Saliva samples were processed and divided in different aliquots for further analysis. For peptide analysis, 10 μL of PMSF 0.1 M, 1 μL of pepstatin 1 mM and 20 μL of anti-protease cocktail (Sigma P2714) were added to 1 mL of collected saliva. After acidification with 0.2% TFA (in the proportion of 1:1), the supernatant obtained from centrifugation at 8000 ***g*** for 10 min (4 °C), was passed through a 50 kDa filter before further analysis. The same anti-protease cocktail (mentioned above) was added to the other fraction of whole saliva and the supernatant aliquots resulting from centrifugation at 12,000 ***g*** for 30 min (4 °C) were kept at −70 °C until analysis. For zymography, part of the collected whole saliva was immediately separated without anti-protease addition and centrifuged under the same conditions as described. Samples were prepared and analyzed in duplicate. The total protein content was estimated using the DC protein assay kit (Bio-Rad, Hercules, CA, USA).

Liquid chromatography separation of peptides

Liquid chromatography separation of saliva peptides was performed as previously described (Vitorino *et al.*, 2009). Briefly, 2 μg of protein from each sample was separated using an Ultimate 3000 (Dionex, LCPackings, Sunnyvale, CA, USA) onto a 150 mm × 75 μm Pepmap100 capillary analytical C_{18} column with 3 μm particle size (Dionex, LC Packings) at a flow rate of 300 nL/min. A linear gradient of 5% buffer B (85% acetonitrile, 0.04% TFA) to 50% buffer B was run over a period of 45 min. The separation was monitored at 214 nm using a UV detector (Dionex/LC Packings) equipped with a 3 nL flow cell. Using the microcollector Probot (Dionex/LC Packings), and after a lag time of 10 min, peptides eluting from the capillary column were mixed with a continuous flow of CHCA matrix solution (270 nL/min, 2 mg/mL in 70% ACN–0.3% TFA and internal standard Glu-Fib at 15 ftmol) and directly deposited onto the LC-MALDI plates at 20 s intervals for each spot (100 nL/fraction).

MALDI-MS analysis and database searching

The MALDI-TOF/TOF MS analysis was performed using a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems, Foster City, CA, USA), as described by Vitorino *et al.* (2009). A signal-to-noise threshold of 50 was used to select peaks for MS/MS analyses. A fragmentation voltage of 2 kV was used throughout the automated runs. The spectra were processed and analyzed by the Global Protein Server Workstation (Applied Biosystems, Foster City, CA, USA), which uses internal Mascot software (version 2.1.0.4, Matrix Science Ltd, UK) for protein/peptide identification based on peptide mass fingerprints and MS/MS data. Searches were performed against the SwissProt protein database (March 2009) for *Homo sapiens*. A MS tolerance of 30 ppm was found for precursor ions and 0.3 Da for fragment ions, as well as two missed cleavages, N-terminal Gln to pyroGlu, oxidation of Met, N-terminal acetylation and phosphorylation at S, T, and Y as variable modifications. Confidence levels above 95% were used as positive protein identification criteria. In order to estimate the false discovery rate (FDR) a reverse decoy database was created for all Swiss-Prot entries. FDR was calculated as FDR = NR/NF, where NR and NF are the numbers of peptides identified from the reverse and forward database searches, respectively, resulting in values <5% of FDR.

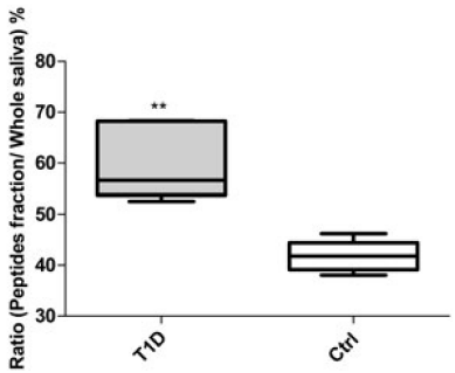

Figure 1. Percentage of saliva peptides to total protein content (**p 0.01). T1D, type 1 diabetes; Ctrl, controls.

Table 1. Contingency table for N-terminal cleavage site differences in identified salivary gland secreted peptides between patients and controls

Percentage	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A						−0.16	0.12						0.02	0.01	−0.07	0.01				
C																				
D						−0.17				0.01	−0.07									
E						−0.63			0.05					0.10	−0.26	−0.08				
F						−0.12	−0.08	0.34					−0.10					−0.46		3.44**
G					0.09	0.66		−0.08	−0.19	0.02		0.05	0.06	−0.21	−0.37					0.66
H	−0.03						0.34						0.05	−0.07	0.41	0.88				
I																				
K	−0.07	−0.03		−0.03	0.23		−0.04			0.02			−0.96		0.40	−0.07				
L	0.27					0.06							0.09							1.30
M													0.02							
N									−0.04			−0.13	−0.17	0.02						0.18
P	−0.19				0,13	0.81	0.05	0.04		−0.20	0.02		1.40	−1.04	−1.40	−0.25				−0.02
Q	−0.16			−0.10		−10.88 *		0.05		0.04			−0.23	−0.01	0.20	0.01				1.65
R	−0.16		−0.05	0.05		−0.37	0.04	−0.85	0.20				−0.01	−0.10	0.00	−0.55				
S	0.28			0.02	0.04	−0.03	0.13		0.05			0.80	−0.24	−0.04	−1.05	0.11		0.02		
T					0.21					0.04			0.04							
V					0.04											0.02				
W																				
Y						2.65			0.31	0.25			0.04	1.33	1.02		0.06			

The amino acids are represented in one-letter codes.
* $p < 0.001$.
** $p < 0.01$.

Cleavage site distribution analysis

For each protein/peptide identified, the corresponding fragments were aligned as a function of the C and N terminus. Residues involved on cleavages at C1–N1 position from the N terminus and at C2–N2 from the C terminus were all identified and aggregated into contingency tables containing counts or frequencies of residues involved in cleavages. These aggregations allow major cleavage site motifs to be sought and give an insight into the proteases involved in the process. The alignment procedure, as well as the cleavage site count, was coded in C# (Microsoft® Visual Studio 2010) using a regular expression-based parser.

Zymography

Zymography assays were performed according to Vitorino *et al.* (2009) with minor alterations. Briefly, 20 μg of protein was mixed with sample buffer and loaded onto 7.5% SDS–PAGE gels impregnated with 0.1% porcine gelatin. After electrophoresis, gels were washed twice for 15 min each in 2.5% Triton X-100 solution and incubated at 37 °C in a developing buffer (50 mM Tris–HCl pH 7.6, 10 mM $CaCl_2$, 10 mM $ZnCl_2$) for 6 h. After incubation, gels were stained with 0.5% w/v CBB G250 for 4 h and destained until clear bands resulting from proteolytic activity were observed. The major bands with gelatinolytic activity were excised from the gel and digested with trypsin. The gel pieces were washed three times with 25 mM NH_4HCO_3–50% ACN, further washed with ACN and followed dried in a SpeedVac (Thermo Savant). Then, 25 μL of sequence-grade modified porcine trypsin (10 mg/mL in 25 mM NH_4HCO_3) were added to the dried gel pieces and samples incubated overnight at 37 °C. Tryptic peptides were extracted by incubation with FA (10% v/v) and ACN (50% v/v), followed by lyophilization in a SpeedVac (Thermo Savant). The tryptic digest were resuspended in 10 μL of a 5% ACN/0.1% TFA solution and separated under the same nano-LC-system conditions used for saliva peptides.

Slot blot analysis

Whole saliva samples were diluted in Tris buffered saline (TBS) to obtain a final protein concentration of 0.001 μg/μL and a volume of 100 μL was slot-blotted into a nitrocellulose membrane (Whatman®, Protan®). The slot blot membranes were blocked with 5% (w/v) dry nonfat milk in TBS-Tween (TBS-T) and then incubated overnight at 4 °C with primary antibodies (anti-MMP-1, anti-MMP-2 and anti-MMP-9) diluted 1:500 in blocking solution. The membranes were washed three times, 10 min each, with TBS-T and incubated for 2 h with secondary antibody (horseradish-conjugated anti-mouse, GE Healthcare, Buckinghamshire, UK) in a dilution of 1:1000. Detection was carried out with enhanced chemiluminescence according to the manufacturer's instructions (GE Healthcare). Quantitative analysis of slot blot was performed with Quantity One software (Bio-Rad).

Statistics

Statistical calculations were performed with the GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego California, USA). Normality of the data distribution was assessed by Shapiro–Wilks normality test. Mean and standard deviation were calculated and a Mann–Whitney test was performed to assess differences between groups in relation to optical density measures.

The statistical comparisons of cleavage site frequency and identified peptides between groups were performed with two-way analyses of variance (ANOVA) followed by the Bonferroni multiple comparison *post-hoc* test. Differences were considered to be statistically significant at *p*-values lower than 0.05.

Partial least squares – discriminant analysis (PLS-DA), is a supervised approach useful for classification and explorative purposes, as it incorporates class membership information (as a **y** vector with dummy values, coded as −1 or 1) in order to maximize class separation. The output consists, among other regression vectors, of scores, where each point represents an individual sample; similar samples will group together in clusters, and a loadings vector reveals the relative contribution of variables (i.e. motifs frequencies) to the positioning of samples on the scores scatter plot (Jackson, 1991).

Results

Salivary peptidome

Unstimulated salivary flow rates ranged from 0.25 to 0.50 mL/min in control individuals, with a mean of 0.35 ± 0.11 mL/min and from 0.20 to 0.50 mL/min in T1D patients, with a mean of 0.34 ± 0.12 mL/min. Despite the similar flow rates, saliva total protein content per minute was calculated and values ranged from 513 to 1294 μg/min in controls and from 456 to 913 μg/min in diabetics. The average of total protein concentration in whole saliva was 2.99 ± 1.48 μg/μL in control individuals and 1.99 ± 0.41 μg/μL in T1D patients. Regarding peptide fractions, the average peptide content was 1.22 ± 0.54 and 1.21 ± 0.35 μg/μL in controls and diabetic patients, respectively. The peptide content corresponded to a higher percentage of saliva total protein content in diabetic patients, representing an average of 60.1% in comparison to 41.8% in the control group, with statistical significant differences ($p < 0.01$; Fig. 1).

From the global analysis of patient and control saliva, 1546 and 989 different peptides, respectively, were unambiguously identified, ranging from 145 to 567 identified peptides (minimum and maximum), with an average number of 447 ± 99 peptides in T1D patients and 272 ± 133 peptides in controls. The identified peptides in controls and T1D patients originated from 200 different proteins (Supporting Information, Table 1). As can be observed in Fig. 2, diabetics presented a higher number of identified peptides with the exception of control 1. The identified peptides were divided into two groups, the salivary gland secreted peptides (SGP) and the nonsalivary gland secreted peptides (NSGP). The SGP group comprised all of the peptides known to be secreted by the salivary glands, based on literature, whereas in the NSGP group were placed the remaining peptides resulting mainly from tissue remodeling or destruction, cellular debris and plasma components.

The inter-individual comparison showed a high number of SGP in all studied individuals, corresponding to an average of 54.9% in controls and of 54.3% in diabetics. Among them, nine peptide sequences were presented in all samples, corresponding to salivary acidic proline rich phosphoprotein ½ (aPRP) ($n = 3$) and submaxillary gland androgen-regulated

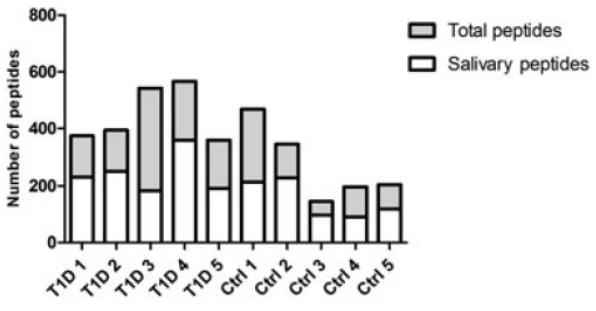

Figure 2. Total identified peptide frequency distribution among type 1 diabetes (T1D) patients and control individuals (Ctrl).

Table 2. Contingency table for C-terminal cleavage site differences in identified salivary gland secreted peptides between patients and controls

Percentage	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A			0.36			−0.36			0.13				−0.02			−0.05				
C																				
D				−0.16		−0.27									0.05	0.32				
E				0.04		−0.10			0.73	0.03				0.05	−0.19	−0.13				0.33
F				0.11		0.42	1.34	0.07		0.24			0.35					−0.63		0.23
G					−0.14	−0.22	−0.15		0.04	0.03		0.05	−1.20		−0.12					0.03
H				0.79	0.16		1.12						0.17		0.28	1.03				
I			0.24			0.14							0.08	−0.01						
K		−0.10			1.53		1.78					−0.01	−0.34		0.14	−0.04		−0.02		0.72
L		−0.18			−0.24			−0.08				0.01	0.10			0.09				
M																	0.03			
N													−0.37	0.05						
P				−0.03		−0.37	0.08						0.65	−0.72	−0.35	−0.06		0.14		0.13
Q		−0.54	0.07	0.01	0.04	−10.37 *				0.01			−0.05	−0.78	0.07	−1.34				0.28
R		−0.10	−0.02	0.07	−0.02	−0.31	0.17	1.23	1.38	−0.06			−0.50	−0.13	0.60	0.09				
S		0.16	0.13			0.51	0.78						−0.20	−0.48		0.13	0.04	−0.01		
T													0.07							
V													0.34							
W																				
Y						0.84			0.03				−0.29	0.40						

The amino acids are represented in one-letter codes.

* $p < 0.001$.

protein 3B (SMR3B) ($n = 6$). Another four sequences were found in eight of 10 individuals, belonging to basic salivary proline-rich protein 1, basic salivary proline-rich protein 3, aPRP and SMR3B (Supporting Information, Table 2).

The NSGP were characterized by the presence of many fragments of collagen proteins and other structural and extracellular matrix proteins (delphilin, elastin, emilin-1, fibrilin-2 and titin). It was possible to identify 464 and 344 unique collagen peptides in diabetic patients and controls, respectively, corresponding to 39 different collagen chains. The most frequent collagen fragments identified belong to alpha 1 chain of type I, II, III, V and VII collagens and alpha 2 chain of type I, II, V and VIII. In spite of a remarkable inter-individual variation on the peptide species identified, significant differences in the number of fragments of alpha 1 chain of type I collagen were found between groups ($p < 0.001$; Fig. 3).

The identified NSGP, resultant from cellular debris, tissue remodeling or destruction and plasma components, were classified according to their protein precursor associated molecular function based on the PANTHER (Protein ANalysis THrough Evolutionary Relationships) system (http://www.pantherdb.org; Mi *et al.*, 2010). In control subjects, the identified peptides were fragments resultant from 89 different proteins, which according to PANTHER classification, belong to the main functional classes: binding (interaction of a molecule with one or more specific sites on another molecule, $n = 65$), protein binding ($n = 45$), proteins with structural function ($n = 42$), extracellular matrix constituents ($n = 37$), proteins associated with receptor binding ($n = 37$), nucleic acid binding ($n = 21$), most of them with DNA binding functions, transcription factor and transcription regulator activity ($n = 12$) and with catalytic activity ($n = 11$).

In T1D patients, we identified peptides from 172 different proteins. After PANTHER analysis, the identified peptides in diabetic individuals were clustered in the same main classes, with a higher number of proteins in each class. It is worth noting the increased number of proteins with catalytic activity ($n = 34$) as well as the number of extracellular matrix proteins ($n = 45$) and in particular the number of proteins with structural function ($n = 60$), mainly from the collagen family of proteins. In the group of proteins with catalytic functions, there stand out the protease disintegrin and metalloproteinase with thrombospondin motifs 19 from ADAMS family, with hydrolase and metalloprotease activities.

Cleavage site distribution of identified peptides

In order to evaluate salivary proteolysis, namely proteases putative identification and cleavage site frequency, all identified peptides were aligned and the C- and N-terminal ends were labeled using home-made software. The protein cleavage site distribution of the identified peptides was based on contingency tables, one for the N-terminal residues (C1–N1) and the other for the C-terminal cleavages (C2–N2). The analysis of N-terminal cleavages in control individuals (Supporting Information, Table 3) showed that the most frequent cleavage sites were the glutamine–glycine (Gln–Gly, 32.89%), followed by cleavages after proline (Pro-X, 17.43%), glycine (Gly-X, 9.40%), glutamic acid (Glu-X, 6.32%), arginine (Arg-X, 4.62%) and tyrosine (Tyr-X, 4.44%). The cleavages in motifs with an arginine (Arg-X or X-Arg) represented 13.27%. Regarding the C-terminal, the most frequent cleavage was also at Gln–Gly motif, corresponding to 32.53% followed by X-Pro motif (29.63%), Pro-X (15.23%), Gly-X (9.51%) and Arg-X (8.31%) (Supporting Information, Table 4).

Regarding diabetic patient peptide analysis (Supporting Information, Tables 5 and 6), a different cleavage pattern was observed. Given the great number of NSGP identified, the cleavage site frequency was analyzed separately for SGP and NSGP, for each participating individual (Supporting Information, Tables 7–10) and for studied groups (Supporting Information, Tables 11–18). The differences in cleavage site frequency between patients and controls are presented in Tables 1–4, which are based on the contingency tables referred above. The N- and C-terminal cleavage site frequency of identified SGP in T1D patients revealed differences when compared with healthy subjects (Tables 1 and 2). The cleavage site frequency of Gln–Gly motif was lower in the SGP of diabetic patients, with a decrease of about 10.88% in the N-terminal and 10.37% in the C-terminal. These differences were statistically confirmed by a two-way ANOVA test ($p < 0.001$). Moreover, for the N-terminal, T1D patients showed a cleavage increase of 3.44% in Phe–Tyr motif ($p < 0.01$). Changes in Gln–Gly and Phe–Tyr motifs frequency were exclusively associated with SGP. As can be seen in Tables 3 and 4, the N- and C-terminal cleavage site frequency of NSGP revealed differences in T1D patients compared with controls. With regard to N-terminal, changes in Gln–Pro, Leu–Pro, Gly–Ala and Gly–Pro motifs were noticed. Regarding the C-terminal, we observed differences in Val–Pro and Glu–Pro motifs. The cleavage site frequency of Gly–Pro and Leu–Pro motifs in N-terminal showed a tendency to decrease in NSGP of diabetic patients, with a reduction of about 2.68 and 1.87%, respectively. An opposite behavior of cleavages was noticed for Gly–Ala and Gln–Pro motifs, increasing 2.40% ($p < 0.001$) and 1.66% ($p < 0.05$), respectively. The cleavage site frequency of Val–Pro motif in C-terminal of NSGP presented a reduction of 1.84% while the motif Glu–Pro increased about 2.42%, with statistically significant differences ($p < 0.05$ and $p < 0.001$, respectively).

In order to maximize the differences between samples (patients and controls) and hence to characterize which motifs are the most important ones for the discrimination between the two groups, PLS-DA was applied both to the motifs counts of terminals C and N (Supporting Information, Tables 2 and 3). In both cases one can observe a discrimination between the two groups along the first latent variable axis (**t**[1]). These discriminations can be characterized by the loading weights of the first latent variable (**w**[1]) for both models (Figs 4 and 5). The full loading weights vector is not shown as most of them have no relative importance for the separation. However, for

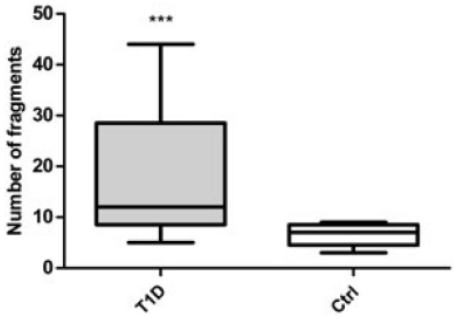

Figure 3. Number of type I collagen alpha 1 chain fragments identified (***$p < 0.001$). T1D, type 1 diabetes; Ctrl, controls.

Table 3. Contingency table for N-terminal cleavage site differences in identified nonsalivary gland peptides between patients and controls

Percentage	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	−0.14	−0.19	0.39	0.12		1.17		0.14	0.21	0.39	0.02	−0.02	0.83	0.35	−0.58	0.16	−0.53	0.12		0.02
C	−0.10		−0.06				−0.11	0.02		0.18		−0.25				0.02	0.03			
D			−0.02			0.75		−0.02	0.19	0.02	0.01		−0.54	−0.36	0.48	0.10		−0.01		0.15
E	−0.66		0.07	−0.92	0.05	−0.30		0.20	0.55	−0.46		−0.21	−0.42	−0.02	−0.03	0.10	−0.37	−0.14	−0.21	
F			0.10	−0.08		0.07		0.40	−0.06	−0.13		−0.43	−0.25	−0.25	0.13	0.03	−0.79	0.02		
G	2.40 *	0.07	0.85	0.59	0.62	0.09		0.24	0.65	−0.48	−0.11	0.22	−2.68*	0.90	0.47	0.16	−0.16	−0.08	0.02	0.00
H		0.13				−0.15	−0.01	0.26	0.12	−0.02	−0.57		−0.22	0.12	−0.14	0.07	0.02			
I	−0.14			0.30	−0.39	−0.44			0.00	0.13	0.07		−0.17	0.18	−0.04	−0.04	0.03			0.25
K	0.61	0.03	−0.20	0.14		0.41			−0.51	0.02	0.14		−1.61	−0.94		−0.05	0.16	−0.08		
L	−0.54	0.10	0.14	0.16	−0.23	−0.59	−0.01	−0.31	0.84	−0.08		−0.31	−1.87**	−0.56	−1.20	−0.40	0.28	0.22		0.06
M	0.13		−0.18			0.30				0.13	−0.07		−0.03	0.15						
N	0.15	−0.03	−0.28		−0.05	−0.06			0.03	0.17	−0.11		0.10			0.06	0.02			
	0.39	−0.17	−0.11	−0.36	−0.21	−1.03	0.25	−0.38	−0.35	0.07	−0.75		0.77	0.03	−0.74	1.54	0.57	−0.81		0.24
Q	0.10	0.02	0.03			1.00		0.02	−0.16	0.07			1.66 **	0.21	−0.05	0.11	−0.02	−0.09	0.02	0.10
R	0.15	0.02	0.01	0.26	0.18	1.24			−0.03	0.08	−0.08	0.10	0.37	−0.17	0.28	0.12	0.21	−0.03	0.20	
S	0.22		0.00	0.09	0.00	−1.51		−0.39	0.10	0.63	−0.03	−0.20	0.28	0.09	−0.01	0.33		−0.03	−0.49	0.18
T	−0.03	0.02	0.20	0.23		−0.13		0.06	0.40	0.12			−0.69	0.03	0.03	0.05	−0.35	0.71	−0.11	0.03
V	0.19		−0.16	0.31	−0.05	−0.15	0.14	−0.05	−0.09	−0.08	0.13	−0.12	−0.07	0.07	0.18	−0.18	−0.27	0.06	−0.28	0.06
W				−0.10												0.03				
Y	0.09		0.04	0.02		0.01		0.15	−0.10				0.10	−0.25	0.20					

The amino acids are represented in one-letter codes.

* $p < 0.001$.

** $p < 0.05$.

Table 4. Contingency table for C-terminal cleavage site differences in identified nonsalivary gland peptides between patients and controls

Percentage	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	0.51					−0.25	0.28		−0.09	0.03			0.14	−0.78	−0.50	0.20		0.14		
C						−0.25			0.15				−0.27							
D	0.17		0.08			−0.70		1.23	−0.39	−0.25		0.00	−0.92	−0.38	−0.17		0.18	0.16		
E	0.02			0.32		0.15	0.07	−0.25	−0.20	−0.32		0.49	2.42*	−0.03	0.09			−0.07	0.28	
F						0.16	0.02	0.03	−0.11			0.06	0.37		−0.54		0.03			
G	0.02		0.09		−0.10	−0.14	0.28	0.15	−0.86	0.30		−0.36	−0.50	−0.08	0.57		−0.18	0.01		−0.21
H					0.10			0.02					−1.09	−0.43				0.17		
I				0.04		−0.50		0.03	0.07	0.18		0.17	−0.43	0.02				0.02		
K				0.02		−0.23	0.17	−0.44	−0.01	−0.19		0.15	−0.58		0.14	0.06		−0.24		
L						0.14	0.29	−0.06	−0.24	0.29		−0.05	1.32		−0.06	−0.20		0.06		
M			0.20	−0.09		−0.35			0.18				−0.85	0.03	0.07					
N						−0.54			0.10	−0.06		0.13	−0.20	0.02		−0.11		0.23		
P	0.59	0.05	−0.03	−0.12		−1.70	0.06	−0.21	0.40	0.37	0.03	0.07	0.07	0.46	0.20	−0.03		0.00		
Q	0.13			0.02		0.36		−0.06	0.14	0.06			1.76	−0.15	−0.11	0.11		0.12		
R	−0.09				0.03	−0.02			−0.04			−0.21	0.53		0.05	−0.01				
S	0.25				−0.09	−0.15				−0.06		0.04	1.40	−0.03	0.18		−0.21	−0.08	0.03	
T						−0.15		−0.08	0.11	−0.06			0.27	0.17	−0.13	−0.11		0.23		
V	0.13				0.02	−0.19	0.18	0.13		0.13		0.17	−1.84**	0.20	−0.05			−0.15		
W									−0.24				−0.01							
Y						0.10						−0.25	−0.52					0.07		

The amino acids are represented in one-letter codes.
* $p < 0.001$.
** $p < 0.05$.

Table 5. Most important motifs identified by PLS-DA models

Terminal N	Terminal C
Gln–Gly [a,b]	Gln–Gly [a,b]
Phe–Tyr[c]	Glu–Pro[c]
Gly–Pro [a,b]	Pro–Gly[b]
Tyr–Gly[c]	Lys–His[c]
Gly–Ala[c]	Gly–Pro [a,b]
Lys–Pro[b]	Gln–Pro[c]
Pro–Pro[c]	Phe–His[c]
Pro–Arg[b]	Lys–Phe[c]
Leu–Pro [a,b]	Leu–Pro [a,b]
Gln–Tyr[c]	Val–Pro[b]

[a]Common motifs for both terminals
[b]most frequent in control individuals
[c]most frequent in type 1 diabetes patients

interpretation purposes the 10 most important motifs for the observed separations are shown in Table 5, sorted by decreasing order of importance of the absolute loadings weights value. Multivariate analysis showed results consistent with the ANOVA described above, presenting the motifs Gln–Gly, Phe–Tyr, Val–Pro and Gly–Pro as the major contribution for the discrimination between controls and T1D patients.

Proteolytic activity evaluation

Zymography analysis revealed the presence of higher intensity bands in diabetic patients in comparison with controls, suggesting an increased proteolytic activity (Fig. 6A). The densitometry evaluation (Fig. 6B) showed four common bands in the zymogram of controls and diabetics, with molecular weights (MW) higher than 250 kDa (band 1), and higher than 100 kDa (band 3), with approximately 92 kDa (band 4) and one band between 50–37 kDa (band 8). Diabetic patients presented other proteolytic bands, specifically between 150 and 100 kDa (band 2), with molecular mass above 75 kDa (band 5), between 75 and 50 kDa

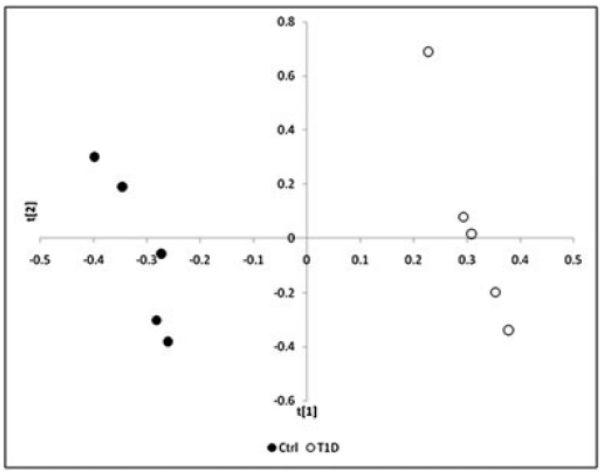

Figure 4. Partial least squares – discriminant analysis scores scatter plot (**t**[1] vs **t**[2]) for the terminal C frequencies; $R^2X[1] = 22\%$ and $R^2y[1] = 97\%$.

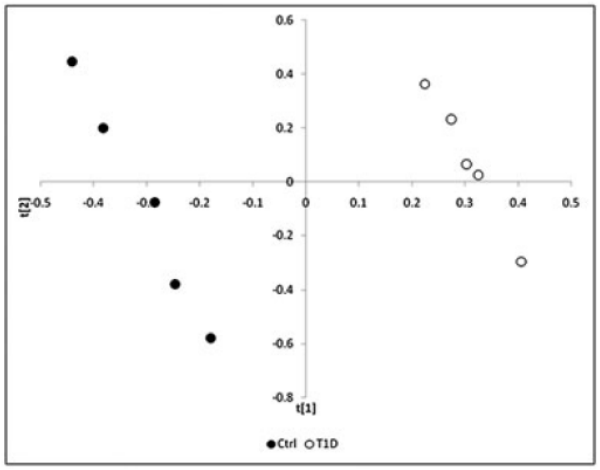

Figure 5. Partial least squares – discriminant analysis scores scatter plot (**t**[1] vs **t**[2]) for the terminal N frequencies; $R^2X[1] = 19\%$ and $R^2y[1] = 94\%$.

(band 6) and between 50 and 37 kDa (band 7). Diabetic patients presented bands with higher optical density in comparison with controls, with statistical significant differences observed for bands 4 and 8 ($p < 0.05$; Fig. 6 C). Using LC-MS/MS for protease identification in each band, only cathepsin D was positively identified in band 8 (Supporting Information, Table 19). The increased proteolytic activity observed in the band 4 from a diabetic's zymogram corresponds to proteases with approximately 92 kDa, the molecular weight of human pro-MMP-9 (Fingleton *et al.*, 2004). Considering also the cleavage site analysis followed by MEROPS (Rawlings *et al.*, 2008) prediction, MMP-2, MMP-3, MMP-12, MMP-13 and, especially, MMP-9, were potentially responsible for the augmented proteolytic activity, which was later confirmed by slot-blot analysis of MMP-1, MMP-2 and MMP-9 to evaluate their levels in whole saliva samples. In the case of MMP-9, higher values were observed in diabetic patients ($p < 0.05$; Fig. 7). For MMP-1 and MMP-2, their presence in saliva was confirmed, although no statistical significant differences were found between groups (data not shown).

Discussion

In spite of the high intra and inter-individual qualitative and quantitative diversity of the salivary peptidome (Le Yondre *et al.*, 2008; Quintana *et al.*, 2009; Vitorino *et al.*, 2009), the inter-individual comparison between all studied subjects showed an overall higher amount of protein fragments in diabetic samples. Peptidomic data (Fig. 2) suggest a higher proteolytic activity in T1D patients, clearly corroborated by zymography analysis (Fig. 6A). In fact, zymography clearly showed bands with higher intensity in the saliva of diabetic patients. Our data is in agreement with previous reports that suggested a diabetes mellitus-related augmented proteolytic activity in the saliva from both humans (Cabras *et al.*, 2010; Kimura *et al.*, 2001) and animal models (Robinson *et al.*, 1997). It is possible to suggest that band 4 corresponds to MMP-9, based on molecular weight and band 8 to cathepsin D, based on MS/MS identification. These bands were the most intense in the zymograms (Fig. 6), which suggests an increased activity of these proteases, confirmed by slot-blot for MMP-9 (Fig. 7). Nevertheless, one should not forget the contribution of other proteases, not identified in the present study,

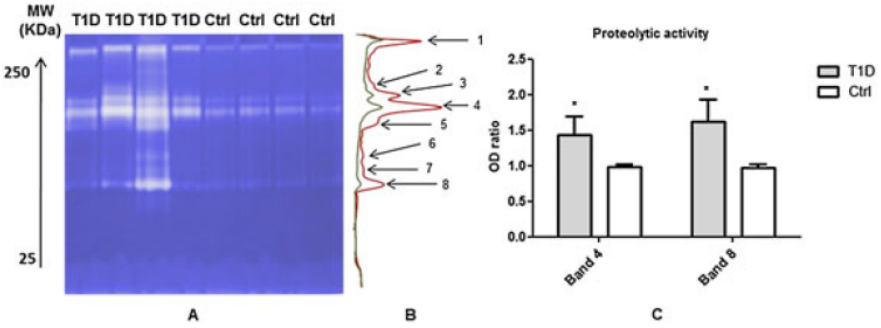

Figure 6. (A) Representative zymography for type 1 diabetes patients and control individuals (Ctrl). (B) An overlap of the average whole-gel lane optical density traces for type 1 diabetes patients (red) and Ctrl (green); (C) optical density measurements of proteolytic bands 4 and 8 (*$p < 0.05$).

to the overall increased proteolytic activity noticed in patients. Our data is in agreement with previous works that reported the presence of MMPs (including MMP-9) as well as cathepsin D in human saliva (Hanemaaijer *et al.*, 1998; Kumar *et al.*, 2006; Sun *et al.*, 2009; Vitorino *et al.*, 2009).

Regarding the cleavage site frequencies, we observed a cleavage increase in diabetics' samples for the motifs Phe–Tyr in SGP and for Gly–Ala, Gln–Pro and Glu–Pro in NSGP (Tables 1, 3 and 4). The observed increase of cleavage on Phe–Tyr motif may be justified by MMP-9 activity or, according to MEROPS (Rawlings *et al.*, 2008), MMP-3, cathepsin G and cathepsin L, well described in human saliva (Brik *et al.*, 2010; Pederson *et al.*, 1995; Sun *et al.*, 2009), and several bacterial proteases. Concerning the Gly–Ala motif, the activity of several MMPs (MMP-2, MMP-3, MMP-9, MMP-12 and MMP-13) might again justify the observed cleavage increase in T1D patients along with other candidates such as cathepsin L and dipeptidyl-dipeptidase 1 and 2, already reported in saliva (Sun *et al.*, 2009). Other cleavage frequencies increased in diabetic patients, namely for Gln–Pro and Glu–Pro motifs. Our LC-MS/MS experimental approach did not succeed well in the identification of the specific proteases responsible for these cleavages. However, based on MEROPS (Rawlings *et al.*, 2008), we might speculate that elastase-2 is responsible for the Gln–Pro motif. This protease is produced by polymorphonuclear leukocytes and is involved in the digestion of extracellular matrix components (Gursoy *et al.*, 2010a; Rawlings *et al.*, 2008). The Glu–Pro motif might result from the activity of aminopeptidase-p produced by oral environment bacteria like *S. mutans* and *S. sanguis* (Cowman and Baron, 1997), well known invaders of the oral environment.

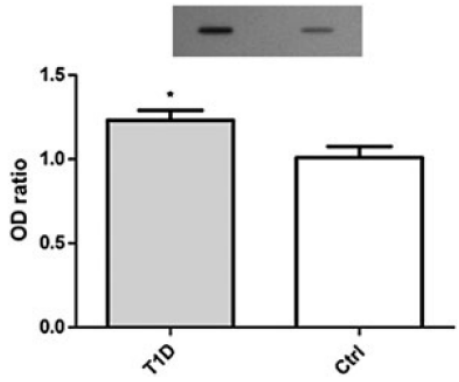

Figure 7. Representative slot blot analysis and optical density measurements for MMP-9 in type 1 diabetes patients and control (*$p < 0.05$).

Many peptides belonging to extracellular matrix and cellular structural components were found in diabetic saliva samples, which may be associated with the well reported high infiltration of inflammatory cells and with the less collagen observed in the histological analysis of gingival biopsies from diabetic patients (Kumar *et al.*, 2006; Lorencini *et al.*, 2009). The expression and activity of MMPs in healthy individuals is in general quite low, significant increases being observed in the same pathological conditions like inflammatory diseases, cancer (Sorsa *et al.*, 2004) and diabetes (Costa *et al.*, 2010; Silva *et al.*, 2008; Varga *et al.*, 2010; Wang *et al.*, 2010). MMPs are capable of degrading extracellular matrix components and modulating immune response by altering activity of cytokines and chemokines by cleavage (Negrato and Tarzia, 2010; Silva *et al.*, 2008). Moreover, Salvi *et al.* (2010) suggested that elevated levels of IL-1 in T1D patients regulates collagen metabolism, mainly through MMP expression (Salvi *et al.*, 2010). In particular, MMP-8, MMP-9 and MMP-13, produced by polymorphonuclear leukocytes and osteoclasts, have been associated with periodontitis and other oral infections, being related to the increase in collagenolytic fragments (Bildt *et al.*, 2008; Gursoy *et al.*, 2010b; Kinney *et al.*, 2007). Our results are also in accordance with previous studies that reported increased salivary levels of MMP-9 in chronic periodontitis patients with diabetes (Kaplan *et al.*, 1978; Kumar *et al.*, 2006). The exacerbation of the inflammatory process and the increased gingival bleeding observed in T1D patients (Javed *et al.*, 2009; Yan *et al.*, 2008) may explain the increased content of proteases in their saliva.

Combining *in-silico* protease prediction, protein identification by mass spectrometry and slot blot analysis, we showed that the main characteristic in the group of NSGP (45% of the total identified) was the presence of a large number of fragments belonging to the collagen family of proteins and other structural and extracellular matrix proteins like delphilin, elastin, emilin-1, fibrilin-2 and titin. Among them, a higher number of fragments from collagen type I was observed in diabetic patients, which may be associated with a chronic inflammatory state, and with an inherent increasing risk of developing oral pathologies, namely gingivitis and periodontitis. In fact, the gingival stroma and the periodontal ligaments are mainly constituted by collagens, type I collagen being the most abundant, and accounting for more than 60% of the total protein. Indeed, a

relation between dissolution of collagen fibers and periodontal inflammation characterizes periodontal disease (Havemose-Poulsen and Holmstrup, 1997).

In conclusion, our results clearly showed a different salivary peptidome profile for diabetic patients. The obtained data strongly suggest that a general higher proteolytic activity in saliva, confirmed by zymography, and in oral tissues leads to an increase in the percentage of peptides and the number of identified peptides in whole saliva of diabetics, which may be, at least partially, explained by the higher activity of the proteases MMP-9 and cathepsin D. It is worth noting that the consistently higher numbers of collagen type I fragments observed in diabetics samples are possibly associated with a chronic inflammatory state of gums and of the periodontal ligaments.

Supporting information

Supporting information can be found in the online version of this article.

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STUDY II -

PROTEASE PROFILING OF DIFFERENT BIOFLUIDS IN TYPE 1 DIABETES MELLITUS

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Protease profiling of different biofluids in type 1 diabetes mellitus

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ABSTRACT

Objectives: We aimed to disclose the proteolytic events underlying type 1 diabetes and related complication through protease profiling in the bodily fluids serum, urine and saliva.

Design and methods: Zymography followed by LC-MS/MS was performed for protease identification and quantitative comparison of proteolytic activity between healthy, type 1 diabetic patients with no complications and with retinopathy and nephropathy. Western blotting was also accomplished for MMP-9 and MMP-2 identification and expression analysis.

Results: Only MMP-2 and MMP-9 were observed in serum with significantly increased levels and activity observed in diabetic patients. In urine and saliva other proteases besides MMPs were identified by MS and presented disease-dependent activity variations. Among these are complex MMP-9/Neutrophil gelatinase-associated lipocalin, aminopeptidase N, azurocidin and kallikrein 1 with more activity noticed in type 1 diabetes patients with nephropathy and/or retinopathy.

Conclusion: Our data highlight the usefulness of urine and saliva for the monitoring of type-1 diabetes-related proteolytic events, where aminopeptidase N, azurocidin and kallikrein 1 appear as promising screening targets for type 1 diabetes-related complications.

Introduction

Diabetes mellitus (DM) is a chronic debilitating disease affecting over 366 million of people worldwide [1,2]. Chronic hyperglycaemia is the critical factor for the development and progression of microvascular complications like nephropathy, retinopathy and peripheral neuropathy in diabetes [3–6]. Nevertheless, the molecular mechanisms influencing the severity of diabetic microvascular disease are not fully understood. Proteomic profiling of clinical species has been heralded as a powerful tool for the identification of altered biochemical pathways and biomarkers of disease states. More than 50 distinct proteins were already suggested as potential biomarkers in bodily fluids from DM patients. Alterations in the levels of distinct apolipoproteins (e.g. A-1, J, C-1) were reported in the plasma and urine of type 1 and type 2 DM, whereas Zn-α-2-glycoprotein 1 and transthyretin were found up- and down-regulated, respectively, in the urine of type 2 DM [7–10]. However, little emphasis has been given to protease profiling in biofluids [11] and even less to its alterations in diabetes mellitus. Besides metalloproteases (MMPs) and kallikreins, no other proteases have been implicated in the pathophysiology of DM and related complications [12,13]. The increased serum levels of these zinc endopeptidases capable of degrading all the components of the extracellular matrix, particularly of MMP-9 and MMP-2, were already suggested as a marker of chronic kidney disease's risk [13] and of active retinopathy [14]. Recently, McKittrick et al. [15] suggested urinary MMP activities as clinically relevant biomarkers for predicting vascular remodeling in diabetic renal and vascular complications in type 1 DM patients. In type 2 DM, kallikrein 3 was found down-regulated in urine [10]. No other proteases have been related to DM pathogenesis though more than 500 human proteases are included in the degradome database and some have been suggested to have context-dependent disease roles [16].

In order to evaluate type 1 DM-related alterations on biofluids' protease profile we performed a straightforward screening of the proteases present in saliva, serum and urine from diabetic patients with no complications diagnosed, with nephropathy and with retinopathy using zymography-LC-MS/MS.

Material and methods

Patients

Subjects enrolled in the present study included 15 type 1 diabetic patients: 5 with retinopathy and nephropathy (group A), 5 with retinopathy (group B) and 5 without chronic complications (group C)

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followed-up by the internal medicine service of Figueira da Foz Hospital, Portugal. Five healthy volunteers (group D) were also included in the study. As inclusion criteria for diabetic patients was defined a minimum duration of diabetes of 10 years. The patients were all examined by the same internal medicine physician. The patients with nephropathy presented a urinary albumin excretion of more than 300 mg in a 24-h collection and retinopathy was diagnosed by an ophthalmologist (Supplementary data, Table S1). The protocol was approved by the Hospital Ethical Committee and followed the Helsinki Declaration. All subjects included in the study gave their written informed consent after being informed of research project's nature. Blood, saliva and urine samples were collected from each individual.

Sample collection

Venous blood samples collected from each subject and glycated hemoglobin (HbA1c) were measured by ion-exchange chromatography using a commercial kit (BioSystems). Blood samples were centrifuged at 3500g for 10 min and serum was stored at −70 °C until analysis. The total protein concentration was evaluated using the LQ total protein kit (Cormay) in automated clinical analyzer Prestige 24 (Tokyo Boeki Medical System).

Unstimulated whole-saliva was collected from all subjects who had refrained from eating and drinking for at least 2 h (performed between 9:00 and 12:00 a.m.), by direct draining into an ice-cold saliva collection tube. Saliva samples were centrifuged at 12,000g for 30 min (4 °C) and the supernatant stored at −70 °C until analysis. The total protein content was determined with the DC protein assay kit (Bio-Rad).

A midstream urine collection was performed and the abnormal presence of leukocytes was screening with urinalysis test strips (Uritest Makromed M10). Urine samples were centrifuged at 1000g for 10 min (4 °C) and the supernatant was passed through a 10-kDa filter to concentrate samples before further analysis. Total protein content was estimated in the fraction corresponding to the retentate using the DC protein assay kit (Bio-Rad).

Zymography and protease identification by LC-MS

Zymography assays were performed as previously described by Vitorino et al. [17]. Briefly, 10 μg of protein was separated by 10% SDS-PAGE. Gels were copolymerized with porcine gelatin. After electrophoresis, gels were washed with 2.5% Triton X-100 solution and incubated at 37 °C in developing buffer (50 mM Tris–HCl pH 7.6, 10 mM $CaCl_2$, 10 mM $ZnCl_2$) for 16 h. Then, gels were stained with 0.5% w/v Coomassie Brilliant Blue (CBB) G250 for 4 h under agitation and destained with 40% methanol/10% acetic acid. In parallel, EDTA (10 mM) and phenylmethylsulfonyl fluoride (PMSF) (5 mM) were included separately in the zymogram developing buffer. Images were acquired using GelDoc XR system (Bio-Rad) and processed using Quantity One® Software (Bio-Rad).

To identify the proteases present in each zymo gel's band, nano-LC-MS/MS was performed. So, bands were excised from the gel and digested with trypsin. The tryptic digest was resuspended in acetonitrile/formic acid solution and separated in a nano-LC-system. LC separation of tryptic digest was performed according to Vitorino et al. [17]. Protein digests were separated using an Ultimate 3000 (Dionex, LCPackings) onto a 150 mm×75 μm Pepmap100 capillary analytical C18 column with 3 μm particle size (Dionex, LC Packings) at a flow rate of 300 nL/min. A linear gradient of 5–50% Buffer B (85% acetonitrile, 0.04% trifluoracetic acid) was run over a period of 45 min. Peptides eluting from the capillary column were mixed with CHCA matrix solution and directly deposited onto the LC-MALDI plate for MALDI-TOF/TOF MS using 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems). An S/N threshold of 50 was used to select peaks for MS/MS analyses. The spectra were processed and analyzed by the Global Protein Server Workstation (Applied Biosystems,), which uses internal Mascot software (v.2.1.0.4, Matrix Science Ltd) for peptide/protein identification based on peptide mass fingerprints and MS/MS data. Searches were performed against the SwissProt protein database (March 2009) for *Homo sapiens*. A MS tolerance of 30 ppm was found for precursor ions and 0.3 Da for fragment ions, as well as two missed cleavages. The confidence levels accepted for positive protein identification were above 95%.

Immunoblotting analysis

Slot blot analysis was performed according to Caseiro et al. [18]. Briefly, saliva and urine samples were diluted in Tris buffered saline (TBS) to a final protein concentration of 0.01 μg/μL and slot blotted into a nitrocellulose membrane (Whatman®, Protan®). For western blot analysis, samples were subjected to electrophoresis (12.5% SDS-PAGE), followed by blotting onto a nitrocellulose membrane (Whatman®, Protan®). The membranes were then incubated with primary antibodies (anti-MMP-2 (clone 101721) and anti-MMP-9 (clone 36020) from R&D Systems) and secondary antibody (horseradish-conjugated anti-mouse, GE Healthcare). Detection was carried out with enhanced chemiluminescence according to manufacturer's instructions (GE Healthcare). Film images were acquired using GelDoc XR system (Bio-Rad) and quantitative analysis of optical density (OD) was performed with Quantity One® 1-D Analysis Software (Bio-Rad).

Statistics

Statistical calculations were performed with the GraphPad Prism version 5.0 for Windows (GraphPad Software). Mean and standard deviation were calculated and one-way analysis of variance or a Kruskal–Wallis test was performed to analyze the statistical significance of differences between groups in relation to OD measures, followed by the Bonferroni or Dunn's multiple comparison post-hoc tests. Differences were considered statistically significant at $p<0.05$.

Results

Analysis of human biofluids' proteolytic profile

In order to screen the proteases present in the most abundant human biofluids and to compare their proteolytic profile, the gelatinolytic protease activity of serum, saliva and urine from healthy individuals was evaluated. As shown in Fig. 1, these fluids contain several proteases capable of hydrolyze gelatin but presented distinct proteolytic patterns. An overlap of the average optical density (OD) for each zymo gel lane corresponding to serum (S), saliva (Sa) and urine (U) is presented in Fig. 1 with the gel bands sequentially annotated with numbers from the lowest to the highest molecular weight. The analysis of serum gelatinolytic proteases' profile revealed one zymo gel band with approximately 72 kDa (band 7) with prominent activity and three others of approximately 86, 92 and 225 kDa (bands 8, 9 and 12) with lower proteolytic activity. Considering the molecular weight, one might suggest the presence of pro-MMP-2, active MPP-9, pro-MMP-9 and pro-MMP-9 homodimer on these zymo bands. This assumption was supported by the complete proteolytic inhibition observed after incubation of zymo gels with 10 mM EDTA (Fig. 3D). Regarding urine, zymo bands with proteolytic activity presented lower intensity in comparison with serum. The analysis of zymogram profile suggests the presence of proteases with approximately 72, 82 and 225 kDa. The molecular weight of the band with higher proteolytic activity in urine (72 kDa; band 7) seems to correspond to pro-MMP-2. Saliva zymography revealed a completely distinct proteolytic pattern with several bands with high proteolytic activity, corresponding to proteases with approximately 43 (band 3), 53 (band 4), 82 (band 8), 92 (band 9), 130 and 225 kDa. Minor activity was detected for proteases' bands with molecular weights

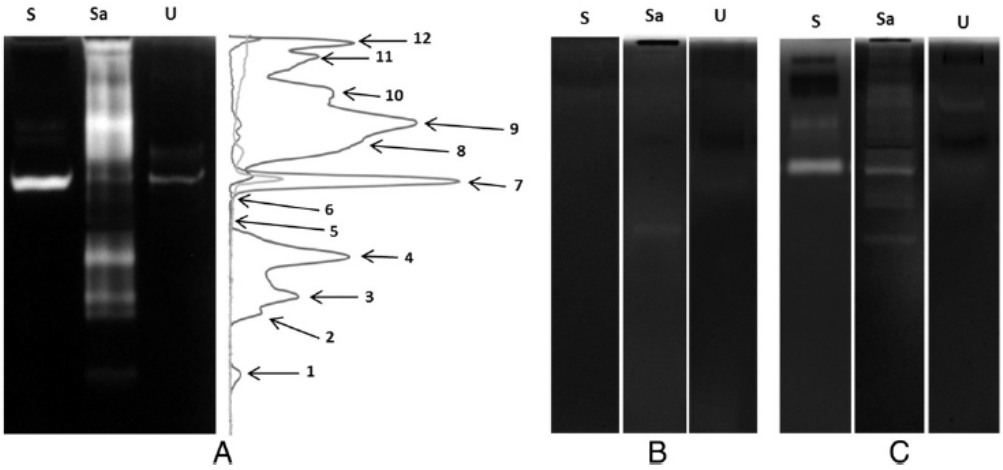

Fig. 1. (A) Representative zymography for healthy individuals and an overlap of the average whole-gel lane OD traces for serum (S), saliva (Sa) and urine (U). (B) Representative zymography for S, Sa and U developed in a buffer with 10 mm EDTA. (C) Representative zymography for S, Sa and U developed in a buffer with 5 mm PMSF.

lower than 30 kDa and with 40 (band 2), 60 (band 5), 66 (band 6), 72 (band 7), and 160–200 kDa (band 11). Considering the complete proteolytic inhibition observed after incubation of zymo gels with EDTA (Fig. 1B) and western blot analysis for MMP-2 and MMP-9 (Fig. 4D), band 6 was assigned as MMP-2, band 7 as pro-MMP-2, band 8 as MMP-9 and band 9 as pro-MMP-9. According to literature [19] and based on western blot analysis, the bands with 130 kDa (band 10) and 225 kDa (band 12) correspond to the complex MMP-9/Neutrophil gelatinase-associated lipocalin and to MMP-9 homodimer, respectively.

In an effort to identify proteases in these biofluids, the zymogram bands were digested with trypsin and analyzed by LC-MS/MS. The proteases identified in each fluid, either by mass spectrometry or western blot analysis, are presented in Table 1. As expected based on zymo incubated with EDTA, western blot and on literature [20–22], several metalloproteinases were identified in all fluids analyzed, namely MMP-2, MMP-9 and pro-MMP-9. These proteases were only identified by western blotting. Besides these, other MMPs like MMP-8 and aminopeptidase N were observed on saliva and on urine, respectively. Other classes of proteases were identified by MS/MS, mainly serine proteases, which is in agreement with the moderated inhibitory effect of PMSF on zymo activity (Fig. 1C). Most of these serine proteases, like vitamin K-dependent protein Z, involved in immune processes, complement C1r subcomponent-like protein, cathepsin A were recognized on urine. Kallikrein-1 and azurocidin were both detected in urine and saliva and myeloblastin only in saliva. Some aspartic proteases as pepsin A and cathepsin D heavy chain were also identified in urine.

Type 1 diabetes-related proteolytic profile of biofluids

The proteolytic profile of biofluids from type 1 diabetic patients with (A and B) and without (C) chronic complications was analyzed and compared with the one from healthy individuals (D). The 15 non-controlled type 1 diabetic patients included in the study were followed for more than 10 years of disease by the same team of physicians, being totally characterized regarding the associated microvascular complications. Individuals from group A presented retinopathy for approximately 12.6 ± 10.8 years and nephropathy for 4.1 ± 3.3 years, whereas individuals with only retinopathy diagnosed for approximately 7.8 ± 6.1 years were included in group B.

In respect to serum, type 1 diabetic patients' proteolytic profile was similar to the observed for healthy controls. Nevertheless, higher activity was noticed considering zymo bands' OD (Fig. 2). An extra band with low proteolytic activity (band S4) was observed in group C patients' serum, at a molecular weight of approximately 130 kDa. Unfortunately, we were not succeeded in its identification. Band S1's OD was evaluated and in all groups of patients presented higher intensity compared to healthy individuals ($p<0.05$; Fig. 2C). Relatively to band S3, all patients presented higher proteolytic intensity, but only with statistical significance in patients from groups B and C. S1, S2 and S3 bands were assigned as MMP-2, MMP-9 and pro-MMP-9, respectively, based on their molecular weight and on previous studies [13,19]. Two zymo bands of approximately 130 (S4) and 225 kDa (S5) corresponding to complex MMP-9/Neutrophil gelatinase-associated lipocalin and to MMP-9 homodimer [19] were also observed without variation among groups (Fig. 2). Besides MMPs, no other proteases were identified in serum.

The gelatinolytic activity of saliva from diabetic patients showed an increment in several zymo bands' activity (Fig. 3A), being possible to distinguish 9 different bands (Sa 1-Sa 9) (Fig. 4B). From OD analysis, significant differences were observed ($p<0.05$) for bands Sa 6, Sa 7 and Sa 9 (Fig. 3C), identified by western blot as pro-MMP-9, MMP-9 and MMP-9 homodimer, respectively (Table 1). From the analysis of zymo band's activity outstand the significantly higher activity of MMP-9 in type 1 diabetics with complications like retinopathy and nephropathy (groups A and B). Besides MMPs, other proteases identified by MS presented an increased activity in the saliva collected from DM patients with complications, namely kallikrein 1 (band Sa 2) and azurocidin (band Sa 1). Compared to serum, a higher intragroup variability was noticed in saliva, particularly evidenced for kallikrein-1 (band Sa 2) and MMP-8 (band Sa 3).

Regarding urine analysis by zymography (Fig. 4A), seven distinct bands (U1–U7) of approximately 60, 72, 82, 92, 130, 190–200 and 225 kDa, respectively, were identified and presented disease-related activity alterations (Fig. 4C). The bands that correspond to active and pro-form of MMP-9 (U3 and U4) presented a gradual activity increase being higher in the patients with chronic complications, namely retinopathy and nephropathy ($p<0.001$) (Fig. 4C). Indeed, more than 3-fold increase was noticed for U4 in patients from group A (Fig. 4C). The activity of MMP-9 homodimer (band U7) was high in all patient groups ($p<0.001$). Most of the proteases identified by MS presented no activity differences between groups with the exception of complement C1r subcomponent-like protein (band U2), higher in DM patients with complications.

To validate data obtained from the analysis of gelatinolytic activity, slot blot assays were performed for saliva and urine, the samples that presented more bands and higher differences between groups (Fig. 5). Slot blot analysis of MMP-2 in saliva showed a gradual increase in patients but without statistical significance, corroborating

Table 1
Proteases identified in saliva (Sa), urine (U) and serum (S) by western blot and mass spectrometry.

Method	Band	Protease	Acession number	MW (kDa)	Fluid	Comparison between groups A vs. D	B vs. D	C vs. D
WB	S5	Matrix metalloproteinase-9 homodimer	P14780	225	S	↑	↑	↑
	Sa9				Sa	↑↑	↑↑	↑
	U7				U	↑↑	↑↑	↑↑
WB/MS	Sa7	Matrix metalloproteinase-9/Neutrophil	P14780/P80188	130	Sa	↑↑	↑↑	↑
	U5	gelatinase-associated lipocalin			U	↑↑	↑	↑
MS	U5	Aminopeptidase N	P15144	109	U	↑↑	↑	↑
WB	S3	Pro-matrix metalloproteinase-9	P14780	92	S	–	↑↑	↑↑
	Sa6				Sa	↑↑	↑	↑
	U4				U	↑↑	↑	↑
WB	S2	Matrix metalloproteinase-9	P14780	82	S	–	–	–
	Sa6				Sa	↑↑	↑	↑
	U3				U	↑↑	↑	↑
MS	U<30	Mannan-binding lectin serine protease 2 B chain	O00187	76	U	–	↑	–
WB	S1	Pro-matrix metalloproteinase-2	P08253	72	S	↑↑	↑↑	↑↑
	Sa5				Sa	↑	↑	↑
	U2				U	↑	↑	–
WB	Sa4	Matrix metalloproteinase-2	P08253	66	Sa	↑	–	–
MS	U1	Cathepsin A	P10619	54	U	–	–	–
MS	U2	Complement C1r subcomponent-like protein	Q9NZP8	54	U	↑	↑	–
MS	Sa3	Neutrophil collagenase (MMP-8)	P22894	53	Sa	↑	↑	↑
MS	U1	Dipeptidyl-peptidase 1 light chain	P53634	52	U	–	–	–
MS	U1	Vitamin K-dependent protein Z	P22891	45	U	–	–	–
MS	U1	Cathepsin D heavy chain	P07339	45	U	–	–	–
MS	U1	Pepsin A	P00790	42	U	–	–	–
MS	U<30	Cathepsin L1 light chain	P07711	38	U	–	↑	–
MS	Sa2	Kallikrein-1	P06870	29	Sa	↑	↑	–
	U<30				U	–	↑	–
MS	Sa4	Myeloblastin	P24158	28	Sa	–	–	–
MS	Sa1	Azurocidin	P20160	27	Sa	↑	–	–
	U<30				U	–	↑	–

All proteases identified by mass spectrometry were validated with at least two peptides with 95% confidence level.
↑↑: statistically up-regulated; ↑: up-regulated; –: no activity differences were observed.

zymography results. In urine samples, slot blot analysis of MMP-2 showed variability between patients, being the observed differences no statistically significant like those observed in zymography. Regarding MMP-9, a significant rise was observed in urine of all patients in comparison with controls ($p<0.05$), while in saliva only a slight expression increase was noticed in diabetic individuals.

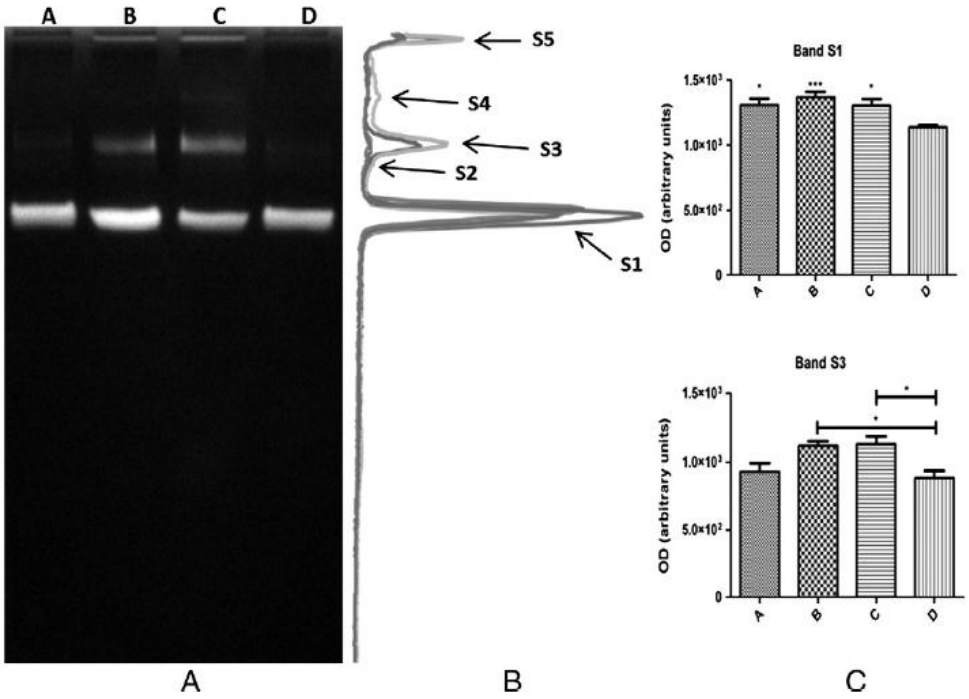

Fig. 2. (A) Representative zymography for serum samples of T1D patients with nephropathy and retinopathy (A), with retinopathy (B), without chronic complications (C) and controls (D). (B) An overlap of the average whole-gel lane OD traces for A, B, C and D serum samples. (C) Optical density measurements of proteolytic bands S1 and S3. *$p<0.05$; ***$p<0.01$.

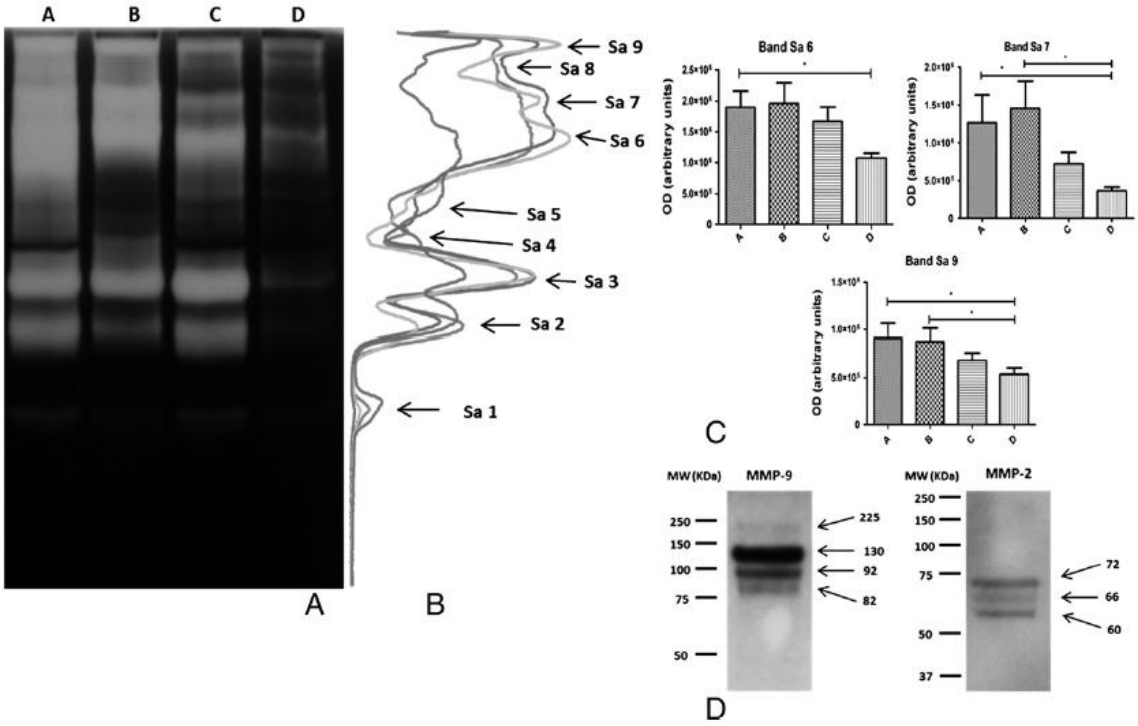

Fig. 3. (A) Representative zymography for saliva samples of T1D patients with nephropathy and retinopathy (A), with retinopathy (B), without chronic complications (C) and controls (D). (B) An overlap of the average whole-gel lane OD traces for A, B, C and D saliva samples. (C) Optical density measurements of proteolytic bands Sa6, Sa7 and Sa9. (D) Western blot analysis for MMP-2 and MMP-9 expression in saliva samples. $^{*}p<0.05$.

These results are in agreement with the ones obtained by zymography, corroborating the good correlation between expression and activity levels of proteases.

Discussion

Proteolysis plays a central role in pathophysiological events, being known for its involvement in inflammatory processes where neutrophils are essential for host defense against invading pathogens. Proteases are part of these cells' array of weapons with, for instance, cathepsin G, elastase and proteinase 3 [23]. Although present in low amounts, these enzymes can produce a high number of protein fragments, modifying not only proteins but also their products yielding a characteristic peptide signature. Serum peptidome analysis already allowed distinguishing metastatic thyroid carcinoma from cancer-free controls based only on its profile [24]. More

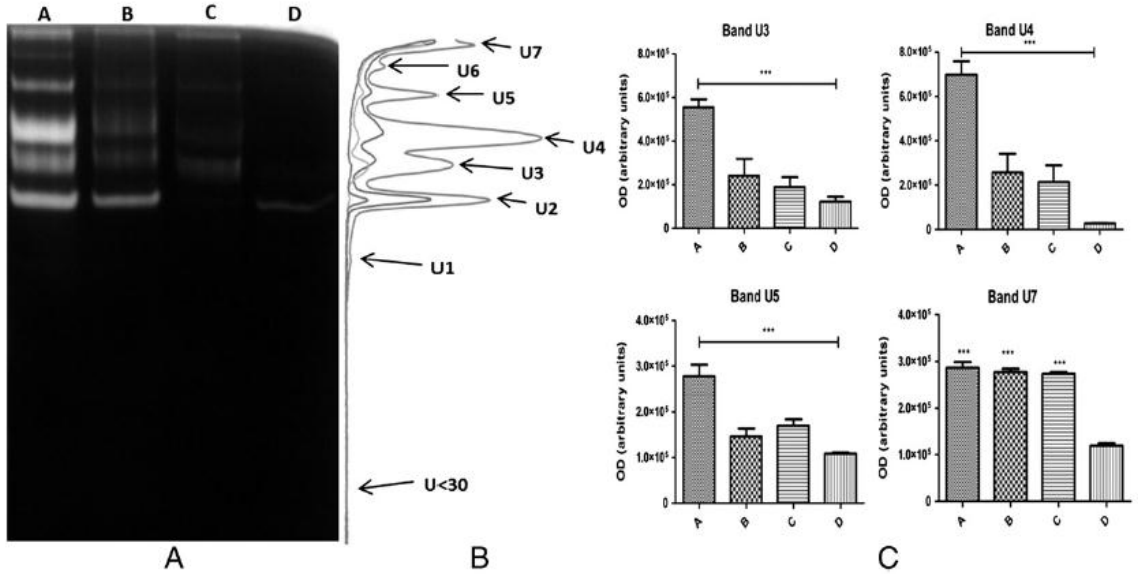

Fig. 4. (A) Representative zymography for urine samples of T1D patients with nephropathy and retinopathy (A), with retinopathy (B), without chronic complications (C) and controls (D). (B) An overlap of the average whole-gel lane OD traces for A, B, C and D urine samples. (C) Optical density measurements of urine proteolytic bands U3, U4, U5 and U7 in samples of T1D patients with nephropathy and retinopathy (A), with retinopathy (B), without chronic complications (C) and controls (D). $^{***}p<0.001$.

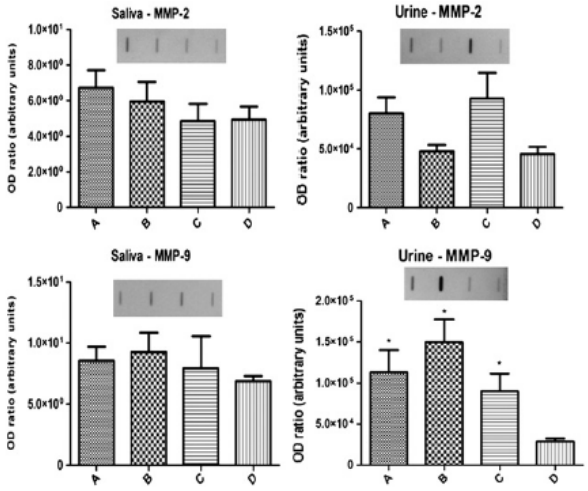

Fig. 5. Representative slot blot analysis and optical density measurements for MMP-2 and MMP-9 in urine and saliva samples of T1D patients with nephropathy and retinopathy (A), with retinopathy (B), without chronic complications (C) and controls (D). *$p<0.05$.

recently, saliva peptidome profiling of type 1 diabetes evidenced several fragments of type 1 collagen as a result of increased activity of MMPs [18]. Although recognized their involvement in pathogenic events, deciphering the activity of proteases *in vivo* is complicated by the presence of other proteins from interactive cascades and pathways [16,25]. In an attempt to disclose the proteolytic events underlying type 1 diabetes' pathogenesis and its related complications, a combined approach with zymography-nano-LC-MS/MS was applied to the bodily fluids serum, saliva and urine. Besides the most used fluids for clinical purposes, saliva was included in the study attending to its increasingly recognized potential for early diagnosis of diseases [26,27], as already tested in oral and systemic pathologies, with some works reporting increased proteolytic activity in periodontitis, Sjogren's syndrome and acute myocardial infarction [28–30].

For the best of our knowledge this is the first study where a deep characterization of proteases underlying type 1 diabetes and related complications like retinopathy and nephropathy was performed simultaneously in serum, urine and saliva. Interestingly, the most outstanding disease-related proteolytic alterations were detected in urine and saliva. Indeed, in serum, less zymo bands were detected comparing to the other fluids. Among the four bands detected, only the ones assigned by western blotting as MMP-9 and MMP-2 presented significantly variations among groups, being higher in diabetics with retinopathy and nephropathy (Fig. 2), as previously reported [12,13].

Disease-related increase of MMPs activity was also observed in the other fluids (Figs. 3 and 4) with a similar variation as observed by others [15,31]. MMPs are known for their involvement in extracellular matrix vascular remodeling and its excretion in urine was already reported in patients with vascular malformation or tumor angiogenesis [15]. McKittrick et al. [15] suggested urinary MMP activities as biomarkers for predicting vascular remodeling in type 1 diabetic renal and vascular complications. Indeed, diabetic nephropathy is the main cause of end-stage renal disease but the mechanisms leading to the development of renal injury are not well defined [32]. More than MMP-9 per se, its complex with neutrophil gelatinase-associated lipocalin (NGAL/MMP-9) was proposed by these authors as an earlier marker of nephropathy, comparing with albuminuria [33]. Our results do not corroborate NGAL/MMP-9 as a specific marker of nephropathy but of type 1 diabetes in general, with and without complications. Thrailkill et al. [34] also described disease- and gender-specific differences in NGAL and MMP-9 concentrations in T1D patients' urine. Although less studied regarding proteolytic events in diabetes, in the saliva of type 2 diabetic patients were also reported disease-related alterations of MMPs activity, more specifically in MMP-9 and MMP-8 [31]. Our results support the contribution of MMP-9 to the previously described salivary peptide signature in type 1 diabetes [18].

While in serum only MMPs were identified, in the other fluids studied more zymo bands were observed and a higher number of proteases (Table 1) seem to contribute to the modulation of biofluids' proteome. Among those identified by mass spectrometry are proteases related with immune functions and biological events as cell proliferation, secretion, invasion and angiogenesis. One of the proteases identified in urine that presented DM-related activity alterations was aminopeptidase N (APN), a type II metalloprotease existent in a wide variety of human tissues and cell types, namely endothelial, epithelial and leukocyte. APN is a multifunctional enzyme related with immune system [35], which according to Mitic et al. [36] present a significantly higher activity in the urine from type 1 diabetic patients with microalbuminuria. Our results support these findings since significantly higher activity levels of this protease were observed in diabetics with retinopathy and nephropathy (Fig. 4, band U5). DM complications-related higher activity levels were also observed for Complement C1r subcomponent-like protein, a serine-type endopeptidase (Fig. 4, band U2) highly expressed in kidney. Its physiological function remains to be determined, but may provide a novel means for the formation of the classical pathway C3/C5 convertase [37]. Although carboxypeptidase cathepsin A was previously identified in the urine of diabetic patients and related to kidney damage [38], no activity differences were found between groups (band U1, Table 1). Kallikrein-1 and azurocidin were identified in urine and also in saliva, with higher activity levels observed in DM patients with retinopathy and/or nephropathy. Previously, azurocidin, a neutrophil granule-derived antibacterial and monocyte- and fibroblast-specific chemotactic glycoprotein, was described as a promising biomarker in gingival crevicular fluid for the development of early diagnosis of periodontitis [39]. Considering that diabetics present a higher risk of periodontitis [39], one might expect to observe increased activity levels of this protease in patient's saliva. The higher activity differences observed by these authors might be justified, at least partially, by the higher concentration of proteases in gingival crevicular fluid (GCF) than in saliva. Moreover, Choi et al. [39] evaluated azurocidin expression levels instead of activity in GCF, which, owing to its very small sample size, submicroliter volumes, and the hard collection procedure associated, make challenging GCF proteome characterization by classical biochemical methods [40]. It also worth of note that though an overall increased proteolytic activity was evident in diabetes' saliva with or without related complications, high variability intra- and inter-groups were observed, highlighting its proteome dynamic nature with potential clinical applications for diagnosis.

Concluding remarks

Overall, our data emphasize the relevance of proteolytic events underlying diabetes and its related complications, which can be monitored in biofluids like serum, saliva and urine. From these, urine and saliva are the ones that present a more pronounced proteolysis' modulation by DM, particularly by its complications. Besides a clear disease-related activation of MMPs was noticed in all fluids, mostly for MMP-9 and its isoform, our data suggest the complex MMP-9/Neutrophil gelatinase-associated lipocalin, aminopeptidase N, azurocidin and kallikrein 1, identified by MS in urine and saliva, as potential screening targets for DM complications. Future prospective studies are essential for the

early predictive value of these potential biomarkers for type 1 diabetes diagnosis and prognosis.

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Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

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STUDY III -

SALIVARY PROTEOME AND PEPTIDOME PROFILING IN TYPE 1 DIABETES MELLITUS USING A QUANTITATIVE APPROACH

Salivary proteome and peptidome profiling in type 1 diabetes mellitus using a quantitative approach

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Running title: Salivary proteomics and peptidomics in diabetes

Keywords: Quantitation, LC-MS/MS, nephropathy, retinopathy, saliva

Under review in Journal of Proteome Research

Abstract

In the present study we applied iTRAQ-based quantitative approach to explore the salivary proteome and peptidome profile in selected subjects with type 1 diabetes, with and without microvascular complications, aiming to identify disease-related markers. From a total of 434 distinct proteins, bactericidal/permeability-increasing protein-like 1 and pancreatic adenocarcinoma upregulated factor were found in higher levels in the saliva of all patients while increased content of other proteins like alpha-2-macroglobulin, defensin alpha 3 neutrophil-specific, leukocyte elastase inhibitor, matrix metalloproteinase-9, neutrophil elastase, plastin-2, protein S100-A8 and protein S100-A9 were related with microvascular complications as retinopathy and nephropathy. Protein-protein interaction network analysis suggests the functional clusters defense, inflammation and response to wounding as the most significantly associated with type 1 diabetes pathogenesis. Peptidome data not only support a diabetes-related higher susceptibility of salivary proteins to proteolysis (mainly of aPRP, bPRP1 and bPRP2) but also evidenced an increased content of some specific protein fragments known to be related with bacterial attachment and the accumulation of phosphopeptides involved in tooth protection.

In overall, the salivary protein and peptide profile highlights the importance of the innate immune system in the pathogenesis of type 1 diabetes mellitus and related complications. This study provides an integrated perspective of salivary proteome and peptidome that should be further explored in future studies targeting specific disease markers.

Introduction

In recent years, saliva has attracted widespread interest as a diagnostic fluid [1-7]. Salivary composition generally reflects the health status of an individual or disease susceptibility for oral and systemic pathologies. The advantages of saliva in comparison with other bodily fluids for diagnostic purposes are given by its accessibility, noninvasive and easy collection. The recent advancements in proteomic technologies hold special promise in the use of saliva to explore novel biomarkers and therapeutic targets [8, 9]. Indeed, new potential diagnostic salivary markers of oral and systemic diseases as dental and gingival pathology, salivary gland disease, Sjögren syndrome, diabetes, head and neck carcinoma, breast and gastric cancers, sclerosis and psychiatric and neurological diseases have been proposed based on proteomic approaches [3, 6-8, 10-12]. Nevertheless, little emphasis has been given to salivary proteome analysis in subjects with diabetes, namely in type 1 diabetes mellitus (DM) [11, 13] and no study is known that quantitatively evaluated the saliva proteome and peptidome changes related with this pathological condition. The importance of quantitative proteomics has been increasingly recognized aiming to provide useful information for clinical applications once it screens non-physiological levels of certain proteins and/or peptides that may reflect pathological conditions [3].

In order to evaluate the effect of more than 12 years of type 1 DM and related complications on their salivary proteome and peptidome, we performed a iTRAQ analysis using pooled saliva samples collected from four groups of individuals (subjects with no diagnosed diabetes-related complications; with nephropathy and retinopathy; with retinopathy; and healthy individuals) to identify potential protein and peptide targets for disease diagnosis. The results obtained highlight the involvement of an inflammatory and immune system response in the pathogenesis of type 1 DM, with higher amounts of proteins like alpha-2-macroglobulin, MMP-9, S100A8 and S100A9 found in the saliva of patients with microvascular complications. Salivary peptidome data not only support a DM-related higher susceptibility of salivary proteins to proteolysis but also evidence an increased content of some specific protein fragments known to be related with bacterial attachment and the accumulation of phosphopeptides that seem to be involved in tooth protection against erosion, more frequent in subjects with diabetes.

Material and Methods

Patients

Subjects enrolled in the present study included 15 type 1 DM patients: 5 with retinopathy and nephropathy (T1D-R+N), 5 with retinopathy (T1D-R) and 5 without chronic complications (T1D) followed-up by the internal medicine service of Hospital Distrital da Figueira da Foz – Portugal. Five healthy volunteers (Ctrl) were also included in the study. All groups were matched by gender and age. Subjects with diabetes presented disease duration of a minimum of 15 years, with HbA_{1c} levels higher than 7.7 %, significantly different from healthy individuals (T1D-R+N vs Ctrl group ($p<0.01$); T1D-R and T1D vs Ctrl group ($p<0.05$)). The patients were all examined by the same clinical team in the management of diabetes. Subjects did not demonstrate evidence of acute and chronic inflammatory disease, infectious diseases, cancer and oral diseases. Smoking subjects were excluded from the present study. The subjects with nephropathy presented a urinary albumin excretion of more than 300 mg in a 24-hour collection and retinopathy was screened by an ophthalmologist. In T1D-R+N, the average duration of retinopathy and nephropathy was 13 and 4 years, respectively. The duration of retinopathy in group T1D-R was of 8 years in average. The demographic and clinical characteristics of enrolled subjects are summarized in table 1.

Table 1: Demographic and clinical characteristics of the subjects enrolled in this study.

	T1D-R+N	T1D-R	T1D	Ctrl
Age (years)	41.31±11.16	46.07±12.28	38.94±13.86	44.75±4.66
Gender (M/F sex ratio)	4/1	3/2	3/2	3/2
Duration of diabetes (years)	29.80±8.80	31.40±11.64	17.40±5.38	--------
Duration of retinopathy (years)	12.6±10.78	7.80±6.14	----------	---------
Duration of nephropathy (years)	4.05±3.35	----------	----------	---------
HbA1c (%)	8.92±1.61 [a]	8.18±0.60 [b]	7.71±0.09 [c]	5.17±0.42
Total cholesterol	205.69±21.95	186.42±27.45	174.20±18.70	177.50±47.74
HDL cholesterol	61.06±7.11	57.74±7.00	64.57±17.49	58.04±4.68

The mean value of each characteristic is presented together with its corresponding standard deviation; a – $p<0.01$ (T1D-R+N vs Ctrl); b – $p<0.05$ (T1D-R vs Ctrl); c- $p<0.05$ (T1D vs Ctrl).

The present study was approved by the Hospital Ethical Committee and followed the Helsinki Declaration. All subjects included in the study gave their written informed consent after being informed of the research project's nature. Saliva and blood samples were collected from each individual.

Sample collection

Unstimulated whole-saliva was collected from all subjects who had refrained from eating and drinking for at least 2 hours (performed between 9:00 and 12:00 a.m.), by direct draining into an ice-cold saliva collection tube. The sample collection time and volume were registered for each individual. Saliva samples were prepared according to Vitorino et al. [14]. Briefly, saliva was mixed with solubilization buffer (7 M urea, 2 M thiourea, 1 % (w/v) CHAPS, 1 % Triton X-100) in the proportion of 2:1. The mixture was sonicated (2 cycles of 5 s each) and then centrifuged at 12000 *g* for 30 min at 4ºC. The supernatant was stored at -70 ºC until analysis. The total protein content was estimated using the DC protein assay kit (Bio-Rad, Hercules, CA.).

Digestion of salivary proteins

For iTRAQ analysis, salivary pools were prepared for each experimental group. Each saliva pool was prepared using equal amounts of total protein from each individual *per* group. The volume of sample pools was normalized for trypsin digestion. Aliquots of 100 µg of protein were analyzed in duplicate. An in-solution digestion was performed for iTRAQ labeling according to the protocol provided by the manufacturer (Applied Biosystems, Foster City, CA). Two independent runs were carried out. Briefly, proteins were reduced, alkylated and digested. Samples were mixed with triethyl ammonium bicarbonate buffer (TEAB) (1 M, pH 8.5) and RapiGest (Waters) to a final concentration of 0.5 M and 0.1 %, respectively and then reduced with 50 mM tris(2-carboxyethyl) phosphine (TCEP) for 1 h at 37 ºC and alkylated with 10 mM S-Methyl methanethiosulfonate (MMTS) for 10 min at room temperature. The aliquots were digested with trypsin (Promega, Madison, WI, USA) at a protein-to-enzyme ratio of 10:1 at 37 ºC overnight and then dried in a Speed-Vac (Thermo Savant, NY, USA).

Peptide labeling with iTRAQs

After protein digestion, the extracted peptides were labeled with iTRAQ reagents (4-plex) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Briefly, one vial of iTRAQ reagent, previously dissolved in 70 µl of ethanol, was added to each aliquot and incubated for two hours at room temperature. The reaction was stopped

by adding water and the labeled digests corresponding to each of the four 4-plex experiments were combined and dried in the Speed-Vac (Thermo Savant, NY, USA).

Peptide separation by multidimensional LC

The tryptic labeled digests were separated by a multidimensional approach based on a first dimension with high pH reverse-phase and a second dimension with the acidic reverse-phase system as previously described [14]. In the first dimension, sample loading was performed at 200 µL/min with buffers (A) 72 mM TEA, 52 mM acetic acid in H_2O, pH 10 and (B) 72 mM TEA, 52 mM acetic acid in acetonitrile (ACN), pH 10 (98 % A: 2 % B). After 5 min of sample loading and washing, peptide fractionation was performed with linear gradient to 50 % B over 35 min followed by a 100 % B step. Sixteen fractions were collected, evaporated, and resuspended in 2 % ACN and 0.1 % trifluoracetic acid (TFA). Collected fractions were separated using an Ultimate 3000 (Dionex, LCPackings, Sunnyvale, CA) onto a 150 mm × 75 µm Pepmap100 capillary analytical C18 column with 3 µm particle size (Dionex, LC Packings) at a flow rate of 300 nL/min. The mobile phases A and B were 2 % ACN 0.1 % TFA in water and 95 % ACN, 0.045 % TFA, respectively. The gradient started at 10 min and ramped to 60 % B till 50 min and 100 % B at 55 min and retained 100 % B till 65 min. The chromatographic separation was monitored at 214 nm using a UV detector (Dionex/LC Packings) equipped with a 3 nL flow cell. The peptides eluting from the column were mixed with a continuous flow of matrix solution (270 nL/min, 2 mg/mL α-CHCA in 70 % ACN/0.3 % TFA and internal standard Glu-Fib at 15 ftmol) in a fractions microcollector (Probot, Dionex/LC Packings) and directly deposited onto the LC-MALDI plates at 12 s intervals for each spot (150 nL/fraction). For every chromatographic run, a total of 208 fractions were collected.

Separation of endogenous salivary peptides

Salivary endogenous peptides were extracted as previously described [15]. To 200 µL of supernantant obtained in sample collection section, ice-cold acetone was added drop by drop in the proportion of 9:1. After agitation on ice for 1 h, the mixture was centrifuged at 19000 *g* for 15 min. The supernatant containing peptides were separated and saved for subsequent analysis. The pellet was resuspended in 200 µL ACN /12 mM HCl, incubated during 1 h on ice under agitation and centrifuged again at 19000 *g* for 15 min. The obtained supernatant was added to the previous one and dried in a Speed Vac for subsequent analysis.

Protein ID and quantification

MALDI-TOF/TOF MS analysis was performed using a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems, Foster City, CA), as described by Vitorino et al [14]. A S/N threshold of 50 was used to select peaks for MS/MS analyses. A fragmentation voltage of 2 kV was used throughout the automated runs. The spectra were processed and analyzed by the ProteinPilot software (v4.0 AB Sciex, USA), which uses paragon algorithm for protein/peptide identification based on MS/MS data. Searches were performed against the SwissProt protein database (release date 01012011) for *Homo sapiens*. Default search parameters were used: specifying trypsin as the digestion enzyme, fixed modification of methylathion on cysteine residue and iTRAQ 4Plex, biological modification with emphasis on phosphorylation and urea denaturation as the variable modification setting. The mass tolerances for precursor and fragments were default values for ProteinPilot®. The cut-off score value for protein identification with ProteinPilot® was a ProteoScore of 1.3 (95% confidence). The false discovery rate at this cut off level is lower than 5%.

Data was normalized for loading error by bias correction, which is an algorithm in ProteinPilot that corrects for unequal mixing when combining the labeled samples of one experiment. It does so by calculating the median protein ratio for all proteins reported in each sample, adjusted to unity and assigning an autobias factor to it. Nevertheless, the quantification results were reviewed manually for all proteins found to be differentially expressed (iTRAQ ratio>1.3 or<0.7 according to Vitorino *et al.* [16]).

Immunoblotting analysis

Slot blot analysis were performed according to Caseiro *et al.* [17] with slight modifications. In brief, saliva samples were diluted in Tris buffered saline (TBS) to a final protein concentration of 0.01 µg/µL and a volume of 100 µL was slot-blotted into a nitrocellulose membrane (Hybond-ECL; Amersham Pharmacia Biotech, Buckinghamshire, UK). The membranes were blocked with 5 % (w/v) dry non-fat milk in TBS-Tween (TBS-T) and then incubated overnight at 4ºC with the primary antibodies anti-deleted in malignant brain tumors 1 protein (Rabbit anti-deleted in malignant brain tumors, P4856Rb, EiAab, Gentaur, Kampenhout, Belgium), anti-cystatin S (Rabbit anti-cystatin S polyclonal antibody, ab58515, Abcam, Cambridge, UK), anti-histatin 1 (Rabbit anti-histatin 1 polyclonal antibody, ab81089, Abcam, Cambrige, UK) and anti-C-reactive protein (Rabbit anti-C-reactive protein monoclonal antibody, ab32412, Abcam, Cambrige, UK) diluted 1:500 in blocking solution and anti-amylase (Rabbit anti-amylase polyclonal antibody,

A8273, Sigma, St. Louis, USA) diluted 1:1000 in blocking solution. The membranes were washed three times, ten minutes each, with TBS-T and incubated two hours with secondary antibody (horseradish-conjugated anti-mouse, GE Healthcare, Buckinghamshire, UK) in a dilution of 1:10000. Detection was carried out with enhanced chemiluminescence according to manufacturer's instructions (GE Healthcare). Film images were acquired using GelDoc XR system (Bio-Rad, Hercules, CA.) and quantitative analysis of optical density (OD) was performed with Quantity One® 1-D Analysis Software (Bio-Rad, Hercules, CA.).

Statistics

Statistical calculations were performed with the GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego California, USA). Mean and standard deviation were calculated and a Kruskal-Wallis test was performed to analyze the statistical significance of differences between groups in relation to OD measures, followed by Dunn's multiple comparison post-hoc test. Differences were considered statistically significant at *p* values lower than 0.05.

Results

Saliva proteome profiling

The merge of two datasets obtained from LC independent runs resulted in the identification of 3834 peptides when based on peptide identifications with ProteinPilot score higher than 1.3 (confidence level of 95 %) and a false discovery rate (FDR) lower than 5 %. In overall, 443 distinct proteins were identified, most of which assigned as presenting catalytic activity (22.6 %) involved in protein binding (30.9 %) and structural molecule activity (17.8 %) according to PANTHER classification system (http://www.pantherdb.org). The mean percent peptide coverage for the complete panel of proteins was 33.7 % (±22.9) with a range of 1.3 % to 89.2 % of coverage. The reproducibility of the experiment was evaluated, and the correlation coefficient was higher than 0.94 in all groups (Supplementary figure S1). Forty four percent of the identified proteins are from different annotated sources besides saliva, like serum and squamous cells.

The relative quantitation of saliva proteins among the four groups (diabetics with no diagnosed complications (T1D), with retinopathy (T1D-R), with retinopathy and nephropathy (T1D-R+N) and healthy individuals (Ctrl)) was firstly evaluated using pools to identify potential protein targets for subsequent confirmation using antibody-based methodologies in all individuals included in the present study. iTRAQ data was analyzed as pairwise ratios: T1D-R+N against the Ctrl (iTRAQ113/iTRAQ116), the T1D+R against the Ctrl (iTRAQ114/iTRAQ116) and the T1D against Ctrl (iTRAQ115/iTRAQ116). Following data analysis, 26 protein candidates (6 % of all identified proteins) were found differentially expressed ($p<0.05$) in diabetics' saliva (T1D-R+N, T1D-R and T1D) compared with control samples (Ctrl) (Supplementary table S1). Figure 1 illustrates the comparison of the log ratio of the relative intensity (T1D-R+N /Ctrl; T1D-R/Ctrl and T1D/Ctrl) to better visualize the salivary proteins differentially modulated by type 1 DM and related complications. As can be depicted from this figure, specific proteins were detected in high levels in all DM patient groups, like bactericidal/permeability-increasing protein-like 1 and pancreatic adenocarcinoma upregulated factor. Other proteins were only found overexpressed in T1D-R+N group, such as alpha-2-macroglobulin, defensin alpha 3 neutrophil-specific, leukocyte elastase inhibitor, matrix metalloproteinase-9 and neutrophil elastase. Proteins like L-plastin variant (fragment), plastin-2, protein S100-A8 and protein S100-A9 were detected in higher levels in the saliva of DM patients with chronic complications (T1D-R+N and T1D-R), though in increased amounts in T1D-R+N patients.

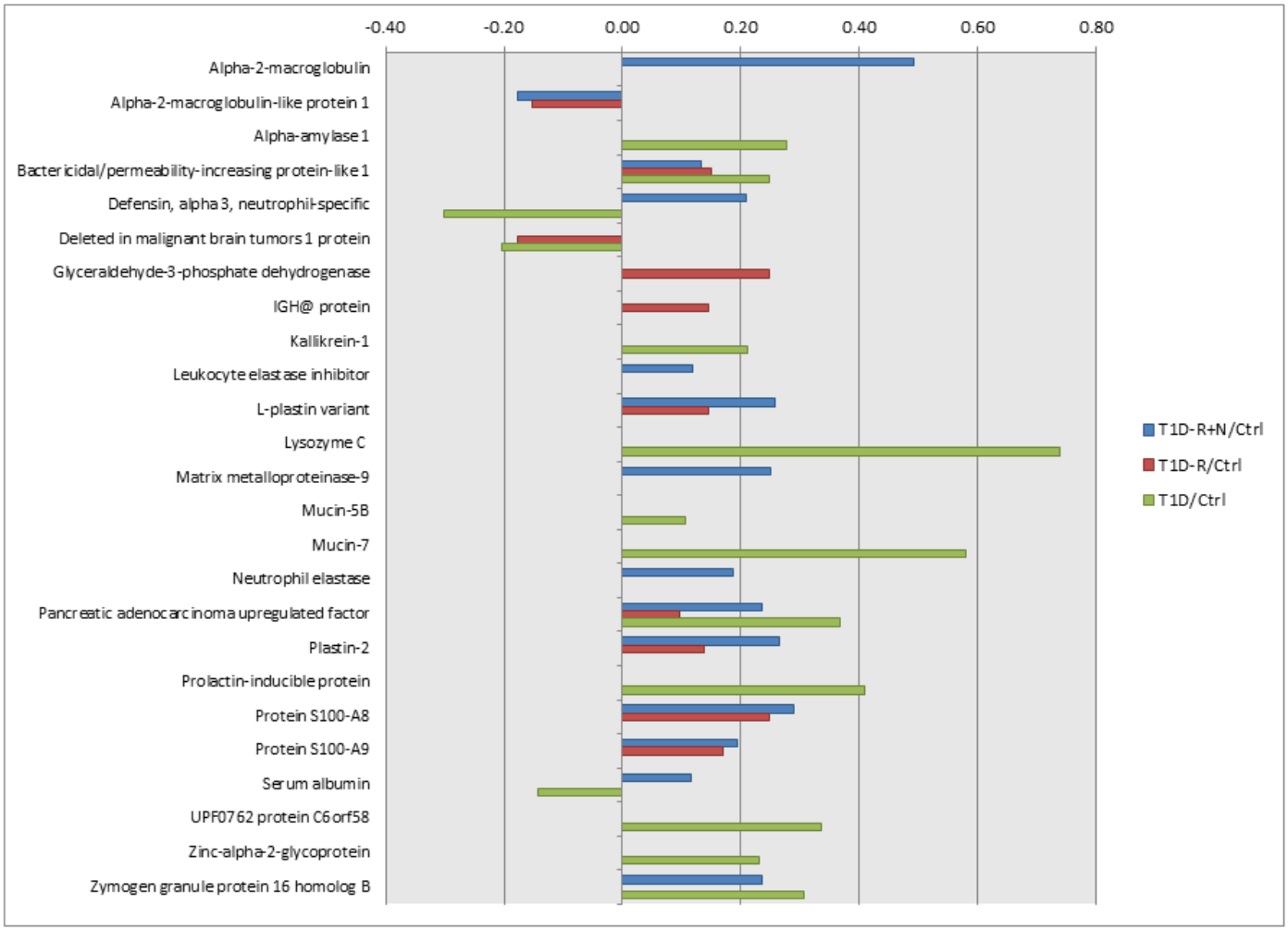

Figure 1: Comparison of the log ratio of the relative intensity of the significantly regulated salivary proteins among groups (T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl).

Looking to the molecular functions and the associated biological processes of these differentially expressed proteins, according to PANTHER (Supplementary figure S2), it can be noticed that the majority are involved in metabolic pathways (23.3 %) and in the immune response (23.3 %). Proteins related to cellular and developmental processes comprise 16.7 % and 3.3 %, respectively, of all DM-modulated salivary proteins. Clusters like cell communication (13.3 %), cellular component organization (3.3 %), response to stimulus (16.7 %) and transport (11.1 %) were also among the ones responsive to DM.

An integrated analysis of all proteins found in different levels among groups was performed with Cytoscape (v2.8.3). The protein-protein interaction network presented in figure 2 was imported from Intact (http://www.ebi.ac.uk/intact/) and contains 266 proteins and 334 protein-protein interactions. These proteins are distributed in ten clusters (Supplementary figure S3), one of which including a downregulated protein, agglutinin (DMBT1), known to interact with proteins involved in proliferation (PARD6B and CDK5RAP3). To identify the relevant biological pathways that were altered in type 1 DM, BiNGO [18] was used to find GO biological pathway and molecular function terms that were enriched among the differentially expressed proteins in the network. In total 576 biological pathway terms and 65 molecular function terms are annotated in association with the differentially expressed proteins. Regarding biological pathways, the most significant ones modulated by type 1 DM are defense, inflammation and response to wounding. Protein binding is the most significant molecular functions found altered in diabetics. Considering the disease-related downregulated proteins, it can be noticed that the majority are involved in binding to bacteria surface.

There are some interactions centered in the overexpressed proteins that are evidenced. For instance, increased levels of alpha-2-macroglobulin seem to be related with alterations in lipid metabolism as evidenced by protein-protein interactions with LCAT, LRP1, leptin and ApoE. This protein is also associated with inflammation as evidenced by its interaction with cytokines as IL-1B and proteins from SERPIN family. The clusters centered in the inflammatory S100 proteins interact with adhesion proteins as VCAM, signaling proteins, namely from the NF-kB pathway, MAP kinase or GTPase mediated signal transduction, and with proteins involved in the regulation of proliferation (e.g. RIF1, CDK2, TP53, GDF9). These S100 proteins are also known to interact with proteins from bacteria and virus as for example glycoprotein B from the human herpesvirus 5. A clear association of the overexpressed PAUF with Toll-like receptors can also be depicted from figure 2. Mostly of the salivary proteins are included in a protein-protein interaction cluster centered in Mucins and amylase.

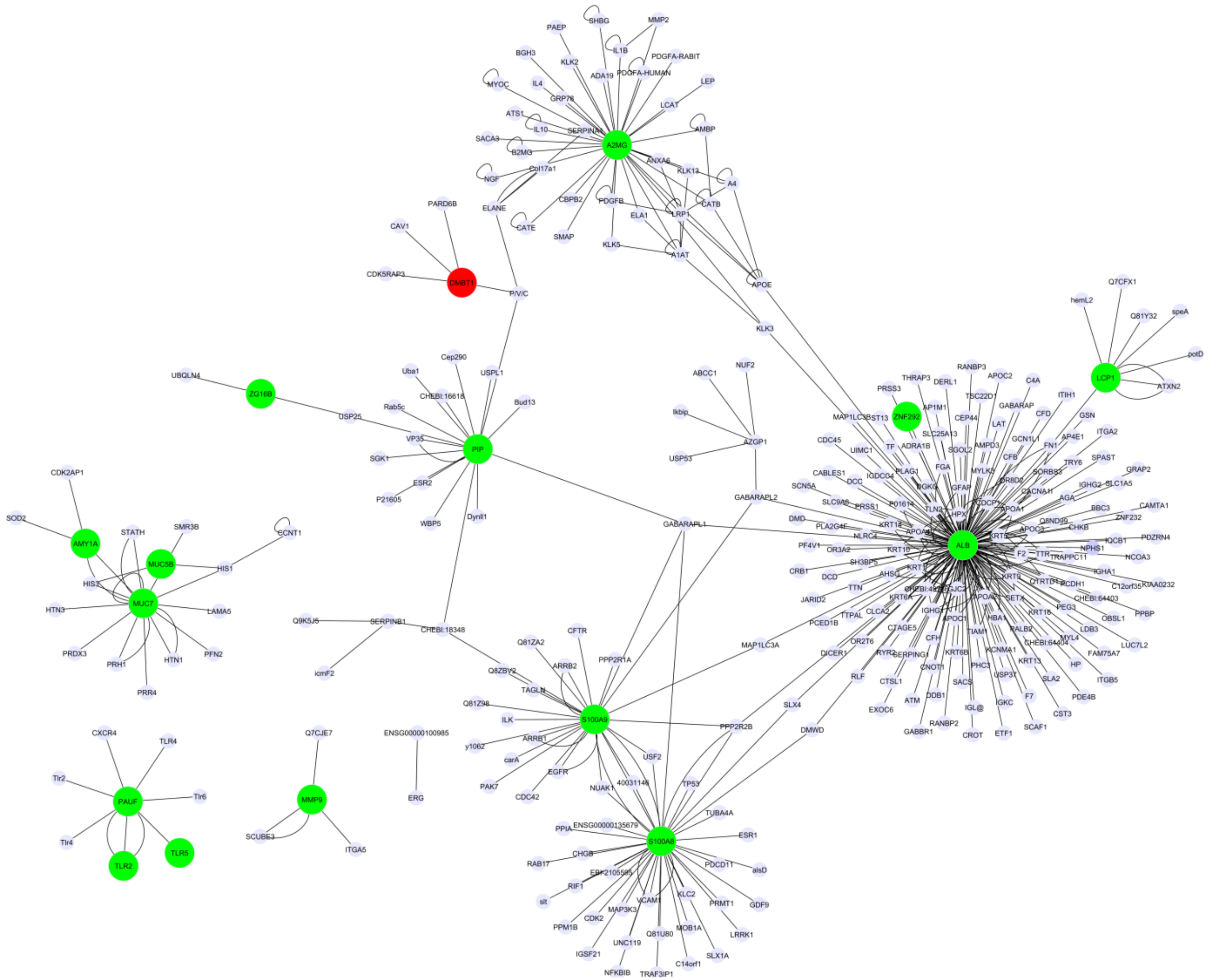

Figure 2: Protein interaction network generated with Intact [56] and visualized with Cytoscape (v2.8.3) consisting of 266 proteins connected by 334 protein-protein interactions. Major clusters of interacting proteins include those involved in defense, inflammation and response to wounding. Green nodes represent upregulated proteins and red nodes downregulated ones in the saliva of subjects with type 1 diabetes.

MUC7, MUC5B and AMY1A interact with statherin, histatins and PRPs. Redox proteins as SOD2 and PRDX3 are also included in this cluster.

For the validation of iTRAQ experiments, immunoblotting analysis of target proteins was performed in individual samples. In this sense, cystatin S, deleted in malignant brain tumors 1 protein and amylase expression were evaluated in triplicate in all saliva samples (from the 15 patients and 5 controls; Figure 3) and data obtained highlight the same tendency observed with iTRAQ analysis. Cystatin S did not present statistical differences between groups as well as in iTRAQ experiment and deleted in malignant brain tumors 1 protein showed DM-related lower values, corroborating iTRAQ data. Increased levels of amylase were observed, in accordance with iTRAQ data, though more significant in T1D-R. C-reactive protein was evaluated in serum samples from the same individuals to screen the inflammatory status. As can be seen in figure 3, significantly higher levels of this inflammatory protein were observed in diabetics with retinopathy and nephropathy.

Saliva peptidome profiling

Saliva contains several protein species of low molecular weight, comprising around 20–30% of the total secreted proteins [19], which contributes to the oral cavity homeostasis. From the LC-MS/MS analysis of endogenous salivary peptides, 794 different sequences from the main salivary protein classes (bPRP1, bPRP2, bPRP3, bPRP4, histatin 1, histatin 3, aPRP, MUC7, PIgR, statherin, SMR3B (P-B peptide) and PROL4) were identified (Supplementary table S2), being aPRP1, bPRP1 and bPRP2 the most representative ones. The distribution of the identified peptides by patient groups can be depicted in figure 4. An average of 270 ± 26 peptides was identified *per* group and from these 182 peptides were common to all groups, 48 were exclusively identified in healthy individuals, 58 in T1D-R+N, 49 in T1D-R and 69 in T1D (Supplementary table S3). Comparing the distribution among groups (Table 2), an increase of bPRP2 was observed in T1D-R+N group in relation to Ctrl (51.50 ± 8.49 vs. 36.25 ± 5.30, $p<0.05$) and a decrease of aPRP peptides in all diabetic patient groups (T1D-R+N, T1D-R and T1D; $p<0.05$, $p<0.001$ and $p<0.001$, respectively). Looking to the unique peptides identified in each group, a predominance of bPRP1 peptides was notorious in diabetics while a higher number of aPRP and statherin unique peptides were noticed in healthy individuals. The alignment of those unique peptides in the main protein sequences evidenced an enrichment of N-terminal peptides from bPRP1 and C-terminal peptides from aPRP in subjects with diabetes (Figure 5). Moreover, the cleavage site motifs were annotated

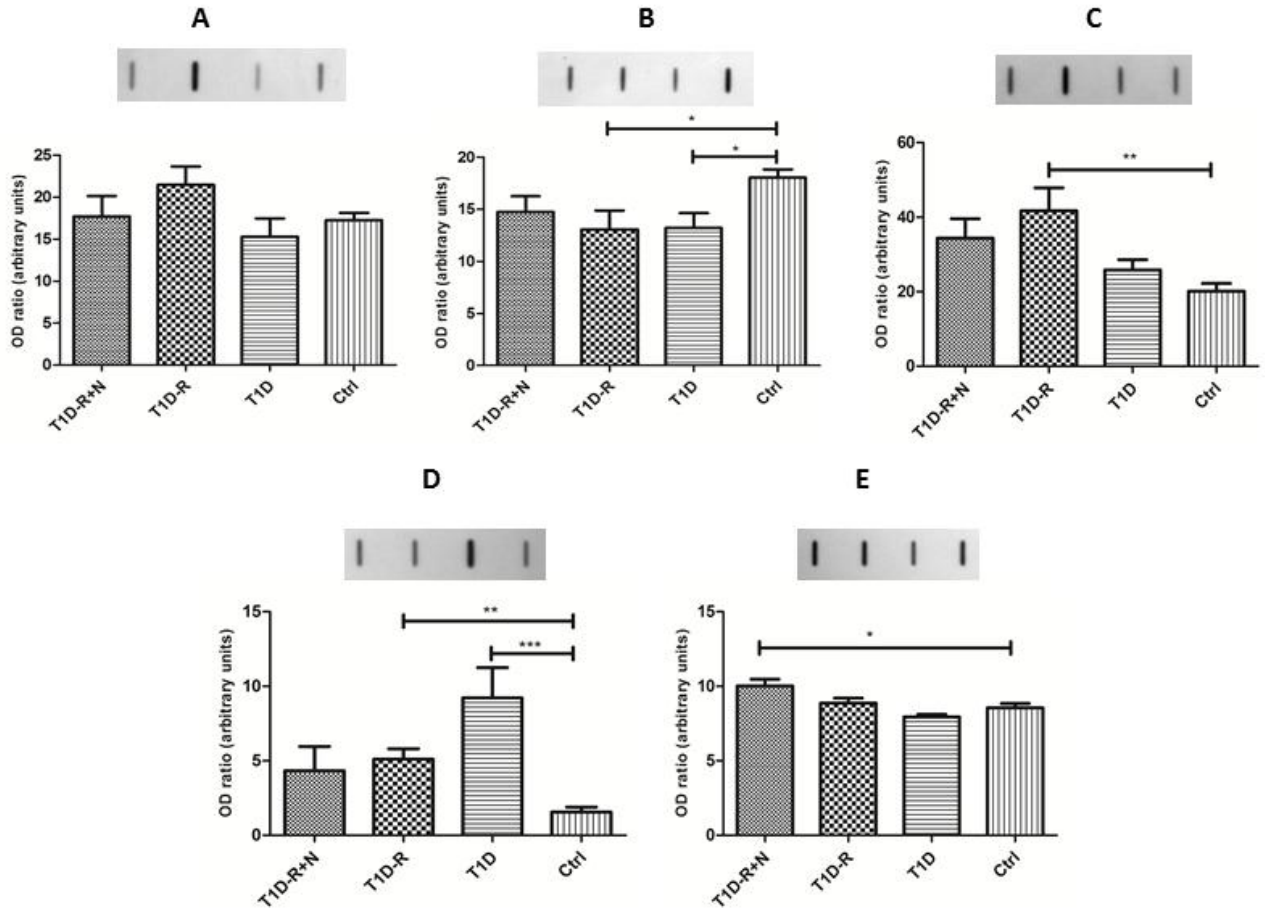

Figure 3: Slot-blot analysis of salivary cystatin S (A), deleted in malignant brain tumors 1 protein (B) amylase (C) and histatin 1 (D). Serum C-reactive protein immunoblotting is presented in (E). Values are presented as mean ± standard deviation of data retrieved from the analysis of individual samples per group. Representative immunoblot images are presented above the corresponding histograms. (*p<0.05 vs Ctrl; **p<0.01 vs Ctrl; ***p<0.001 vs Ctrl).

Table 2: Distribution of the identified peptides *per* group and protein class according to their relative abundance. Data is presented as mean ± STD.

	T1D-R+N	T1D-R	T1D	Ctrl
Basic salivary proline-rich protein 1 (bPRP1)	55.25±11.67	47.50±16.26	55.25±7.42	42.50±4.24
Basic salivary proline-rich protein 2 (bPRP2)	51.50±8.49	35.50±11.31	47.25±7.42	36.25±5.30
Basic salivary proline-rich protein 3 (bPRP3)	15.75±2.47	14.25±4.60	15.75±1.06	19.50±2.83
Basic salivary proline-rich protein 4 (bPRP4)	25.00±4.24	13.00±6.36	10.75±0.35	18.75±1.77
Histatin 1	13.75±6.72	19.00±9.90	12.75±0.35	12.75±1.06
Histatin 3	5.75±0.35	7.25±1.77	10.75±5.30	2.00±1.41
Mucin-7 (MUC7)	8.00±0.71	4.25±1.06	7.50±4.95	7.00±0.71
Polymeric immunoglobulin receptor (PIgR)	1.25±0.35	1.00±0.00	2.75±1.77	1.75±0.35
Proline-rich protein 4 (PROL4)	1.50±0.71	0.75±1.06	0.25±0.35	1.75±1.06
Salivary acidic proline-rich phosphoprotein 1/2 (aPRP)	65.50±13.44	51.25±18.74	56.25±11.67	81.75±10.25
Statherin	27.25±8.13	19.75±9.55	20.25±3.18	30.25±1.06
Submaxillary gland androgen-regulated protein 3B (SMR3B)	29.25±12.37	26.25±8.84	18.25±7.42	29.00±5.66
Total	299.00±69.30	239.75±83.79	257.75±36.42	283.25±21.57

using a home-made software for protease prediction. This analysis highlighted the predominance of Q/G and P/Q motifs in the saliva from diabetics (Supplementary table S4). Looking for potential proteases acting on these motifs, MEROPS (http://merops.sanger.ac.uk) [20] search was performed and retrieved cathepsin L and MMP-2/MMP-9 as the most probable ones for Q/G and P/Q motifs, respectively.

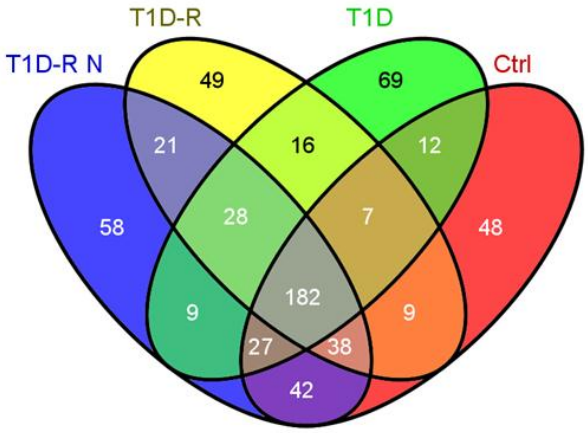

Figure 4: Venn diagram representing the distribution of identified peptides *per* group (T1D-R+N, T1D-R, T1D and Ctrl) evidencing the overlapped and unique peptides.

The presence of specific post-translational modifications such as phosphorylation, Gln->pyro-Glu at N-terminal, oxidation, glycation, glycosylation as well as protein variants were searched in all salivary peptides. From previously identified salivary protein variants ([21, 22], we only observed fragments of PRH2-1, a variant of aPRP, which corresponds to the replacement of Asp by Asn in the position 66 (Supplementary figure S4).

The comparative analysis of these PTMs between groups showed a significant 2.2-fold increase of Gln->pyro-Glu in PROL4 peptides from diabetics in comparison to controls (Supplementary table S5). HexNac modification in bPRP2 was denoted in diabetics with chronic complications in comparison to Ctrl group (2.4-fold in T1D-R+N and 2.5-fold in T1D-R). The HexNac modification in bPRP3 was exclusively observed in T1D-R+N group. The percentage of phosphorylated peptides originated from bPRP3 and aPRP was higher in subjects with diabetes, accounting for 5-fold increase of bPRP3 phosphopeptides in T1D-R+N and T1D groups in comparison to Ctrl ($p<0.001$). The same tendency was observed for aPRP. The higher content of phosphorylated and glycosylated peptides in diabetics reflects the data given by the slot blot analysis of whole saliva using specific

staining methods (Diamond ProQ and Emerald ProQ, respectively) for these modifications (Supplementary figure S5).

In order to evaluate the impact of DM in saliva peptidome, we performed a comparative iTRAQ-based analysis of identified peptides between patients with and without chronic complications and controls (Supplementary figures S6-S9). From all identified peptides above-referred, 99 presented differences among groups (Supplementary table S6). From these, fragments of histatin 1 presented higher levels in T1D and T1D-R. This tendency was also observed by immunoblotting for intact histatin 1 (figure 3D). Statistical significance was observed for 5 distinct peptides (Table 3). For instance, the peptides originated from bPRP3 (^{289}GPPPPPQGGRPHRPPQGQPPQ^{309} and ^{297}GRPHRPPQGQPPQ^{309}) were observed in higher levels in T1D group ($p<0.01$) whereas the peptide ^{297}GRPHRPPQGQPPQ^{309} was only found in increased amount in group T1D-R group ($p<0.05$). The peptide ^{148}GPPPQGGRPQGPPQGQSPQ^{166} from aPRP was detected in higher levels in patients groups T1D-R and T1D ($p<0.001$, Figure 5).

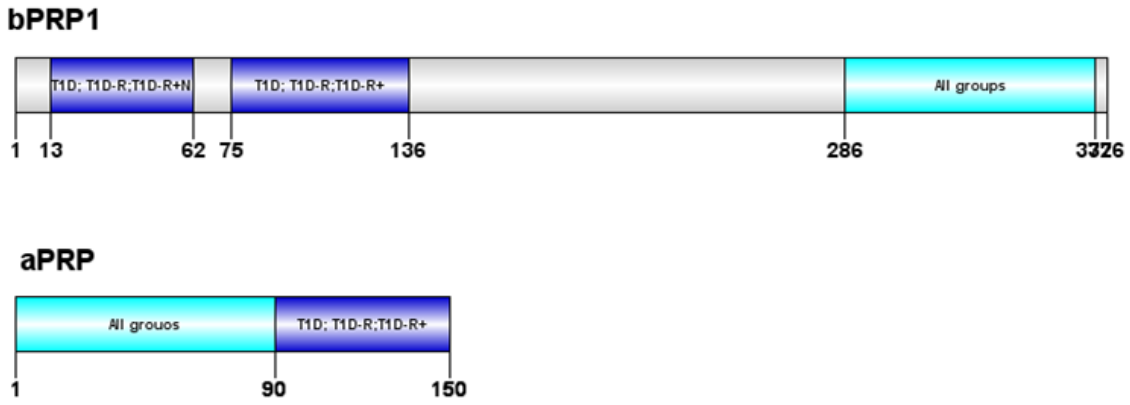

Figure 5: bPRP1 and aPRP main sequences evidencing the location of the unique peptides identified in all groups (light blue) and only in diabetics (dark blue).

Table 3: Peptides presented in significantly different levels between groups identified based on iTRAQ analysis. Data is presented as mean ±STD.

Name	Conf	Sequence	ΔMass	Theor m/z	T1D-R+N /Ctrl	T1D-R /Ctrl	T1D /Ctrl
Basic salivary proline-rich protein 1 (bPRP1) or basic salivary proline-rich protein 2 (bPRP2)							
	98	GPPPQGGNKPQGPPPPGKPQ	0.02	2844.62	5.92±0.03[#]	6.58±0.02[#]	6.64±0.06[#]
	99	GPPQQEGNNPQGPPPPAGGNPQQPQ	0.05	2792.37	4.54±0.02[£]	1.17±0.00	2.18±0.02
Basic salivary proline-rich protein 3 (bPRP3)							
	96	GRPHRPPQGQPPQ	0.00	1755.97	1.41±0.01	1.37±0.00	5.25±0.05[¥]
	99	GPPPPPQGGRPHRPPQGQPPQ	0.07	2483.33	1.68±0.01	6.50±0.02[Ω]	6.77±0.06[‡]
Salivary acidic proline-rich phosphoprotein 1/2 (aPRP)							
	96	GPPPQGGRPQGPPQGQSPQ	-0.02	2171.13	2.10±0.01	11.17±0.03[ϕ]	12.61±0.11[ω]

[#] T1D-R+N, T1D-R and T1D vs Ctrl ($p<0.001$); [£] T1D-R+N vs Ctrl ($p<0.05$); [¥] T1D vs Ctrl ($p<0.01$); [Ω] T1D-R vs Ctrl ($p<0.01$); [‡] T1D vs Ctrl ($p<0.05$); [ϕ] T1D-R vs Ctrl ($p<0.001$); [ω] T1D vs Ctrl ($p<0.05$).

Discussion

To the best of our knowledge, the present study represents the first attempt to determine salivary protein profile alterations in type 1 diabetes mellitus using iTRAQ experiment. The objective of this approach was to compare salivary proteome and peptidome profiles among diabetics (with and without chronic complications) and healthy individuals (control group). Only clinically characterized subjects with type 1 diabetes, with a history of more than 12 years of disease and grouped according to DM-related complications, were selected by physicians for the present study. No other systemic and oral diseases were clinically evident. All subjects with diabetes presented significantly augmented HbA_{1c} levels in comparison to the control group, being these differences higher in patients with retinopathy and nephropathy despiting not significant differences of diabetes duration (Table 1).

From the comparative analysis of saliva proteome performed by LC-MS/MS with iTRAQs, several proteins were identified that could potentially be used to distinguish individuals with type 1 DM as well as the ones with chronic complications associated with this disease. According to PANTHER, the majority of them are involved in metabolic (23.3 %) and immune response (23.3 %) processes, which corroborates the nature of the pathology, *e.g.* a metabolic disorder accompanied with inflammation [23]. This phenotype was further corroborated by protein-protein interaction network analysis of differentially expressed proteins performed with Cytoscape. This analysis highlighted the functional clusters defense, inflammation and response to wounding as the most relevantly modulated by type 1 DM. These findings are in accordance with data retrieved from the integrative analysis of diabetes mellitus-related proteome alterations of biofluids, which suggests the involvement of immune and cellular processes independently of the fluid analyzed [24].

The significant upregulation of bactericidal/permeability-increasing protein-like 1 (BPI) and Pancreatic adenocarcinoma factor (PAUF) in the saliva of all diabetics clearly suggest the activation of the immune system in type 1 DM. BPI is an essential component of the innate immune system with bacteriostatic and bactericidal effects against gram-negative bacteria through lipopolysaccharides binding [25, 26], whereas PAUF is an endogenous ligand of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4) [27]. TLR2 ligand stimulation induces nuclear translocation of nuclear factor-κB through MyD88-dependent intracellular signaling pathway, modulating gene transcription and, consequently, inflammatory cytokine production [28]. The inflammatory response seems more

exacerbated in patients with retinopathy and nephropathy considering the high salivary levels of alpha-2-macroglobulin, defensin alpha 3 neutrophil-specific, leukocyte elastase inhibitor, matrix metalloproteinase-9 and neutrophil elastase. The protease inhibitor alpha-2-macroglobulin and the leukocyte elastase inhibitor were previously reported, based on proteomic data, as being upregulated in the saliva of diabetics but with type 2 DM [12]. Increased levels of alpha-2-macroglobulin were also observed in serum samples from diabetics with complications [29, 30]. The involvement of the naturally occurring inhibitor of neutrophil proteases identified in this study was previously described in several human inflammatory diseases [31]. Chan et al. [32] also found an association between the salivary levels of this protein and periodontitis in subjects with type 2 diabetes. Nevertheless, this correlation was not supported by data obtained from Zhou *et al.* [33]. Despite the increased levels of these protease inhibitors detected in the saliva of diabetics, higher content (*e.g*. MMP-9; Supplementary table S2) and activity of MMPs [34-37], and of neutrophil elastase, which activity is known to be regulated by leukocyte elastase inhibitor [38], have been reported.

Other molecular players of the inflammatory process were found in higher content in the saliva of diabetics, particularly in the ones with complications. The upregulation of the actin bundling protein L-plastin was previously described in subjects with type 2 diabetes [32] and in non-diabetic individuals with periodontitis [39]. The possible association with periodontitis was not corroborated by Grant *et al.* [40] that found lower levels of L-plastin in healthy volunteers with gingivitis. The higher content of S100-A8 and S100-A9 observed in diabetics suggests that in these patients occurs the formation of the heterodimer known as Myeloid-Related Protein-8/14 (MRP8/14), which binds to the receptor for advanced glycation end products and TLR4, mediating intracellular inflammatory signaling transduction [41-43]. Elevated MRP8/14 complex plasma levels was already suggested as a prediction factor for increased risk of cardiovascular events [44] and were associated with inflammatory diseases, especially rheumatoid arthritis [45]. Cabras *et al.* [11] also reported higher levels of S100A9 in the saliva of type 1 diabetic children in comparison with sex and age-matched healthy subjects. But more than a relation with type 1 diabetes *per se*, our data suggest an association between increased salivary content of MRP8/14 and microvascular complications, which were not described before and could be used in the future in the monitoring of diabetes-related chronic complications.

Together with the analysis of salivary proteome, peptidome profiling might give new insights of the effect of type 1 DM and related chronic complications on oral cavity

homeostasis and consequent physiological impairment. In this sense, a straightforward analysis of low molecular weight salivary peptides (<5 KDa) was performed using an iTRAQ experiment for quantitative comparison of peptidome profiles. In overall, a higher content of peptide fragments from the main salivary protein classes, mainly from aPRP, bPRP1 and bPRP2, was observed in subjects with diabetes (Table 2), which seems to be related with the higher proteolytic activity previously reported in the saliva of diabetics [23, 36, 37, 46], being PRP classes the most susceptible ones [46, 47]. Moreover, the predominance of KPQ motif in acidic and basic PRPs [46] might be attributed to a glutamine endoprotease not yet identified, which could be originated from the oral microbiome [48]. However, among the proteases modulated by the disease we identified by iTRAQ analysis MMP-9 as being overexpressed. This data was also corroborated by MEROPS [20] search of more prevalent cleavage sites (Supplementary table S4). The higher activity of MMP-9 as well as of other MMPs was already reported in the saliva of subjects with type 1 diabetes [36, 49]. Protein-protein interaction network analysis also highlight a correlation between the higher levels of MUC and salivary peptides, mainly statherin, histatins and PRPs (Figure 2), suggesting an attempt to maintain the oral homeostasis.

The analysis of the unique peptides in each group evidenced an enrichment of N-terminal peptides from bPRP1 and C-terminal peptides from aPRP in diabetics. Although bPRP role in oral cavity are not well characterized, in the case of aPRP, the C-terminal peptide (^{148}GPPPQGGRPQGPPQGQSPQ^{166}) is known to be associated with bacterial attachment via PQ segments to tooth surface, avoiding bacterial binding [50]. The elevated levels of this peptide together with the high content of BPI detected in the saliva of diabetics might be seen as a protective mechanism against bacterial infection that predispose to tooth demineralization [51, 52]. The elevated content of these peptide fragments was also paralleled by increased proteolytic activity observed in children with diabetes, as previously observed by Cabras *et al.* [11]. According to these authors, the low concentration of the intact P-C peptide associated with high levels of its fragments suggests the impairment of oral cavity safeguard given by salivary peptides. A different tendency was observed for histatin 1 in the saliva of adult subjects (with approximately 40-years old), with DM-related higher content of intact and fragments of this salivary peptide.

In order to get a deep perspective of the peptidome dynamics, other post-translational modifications besides proteolysis were analyzed. Data obtained points to a functional impact of phosphorylation and glycosylation (Supplementary table S5). From those prevalent phosphopeptides, no significant expression differences were detected among

groups using iTRAQ analysis; however, a higher number of phosphopeptides was detected in the saliva of diabetics. In fact, the phosphorylation of serine residues in PRPs, sthaterin and histatin 1 has been associated with the acquired enamel pellicle formation and, consequently, with the participation in the remineralization process, having a protective effect against tooth erosion [53, 54]. A similar trend was also observed for HexNAc modification in bPRPs, which is in agreement with our previous findings for head and neck cancer where a predominance of this modification was detected [21]. Furthermore, the higher levels of HexNac modification might also reflect the *O*-GlcNAcylation observed during diabetes that might act as an autoprotective alarm or stress response [55]. This data is in agreement with the increased expression of pro-inflammatory mediators observed in the saliva of subjects with diabetes, particularly in the ones with chronic complications (Figure 1).

Conclusion

Data from the present study highlight the potential use of saliva as a diagnostic fluid for the monitoring of diabetes and related microvascular complications. Salivary proteome evidenced an overexpression of the endogenous regulators of TLR, MRP8/14 and PAUF, affecting myeloid MyD88-dependent activation of NF-κB and tumor necrosis factor-alpha expression, in the saliva of subjects with diabetes-related retinopathy and nephropathy, pointing to the importance of the innate immune system in the pathogenesis of DM-related complications.

Peptidome data not only supported the DM-related higher susceptibility of salivary proteins to proteolysis but also evidenced an increased content of some specific protein fragments known to be related with bacterial attachment and the accumulation of phosphopeptides that seem to be involved in tooth protection against erosion. Particularly, the proteolytic fragments from bPRP1, bPRP2 and aPRP might be seen as a hallmark of the disease pathogenesis with potential use for its monitoring. Future studies should be undertaken to disclose the functional role of these salivary peptides under different pathophysiological conditions aiming to define disease-specific biomarkers.

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CHAPTER III

GENERAL DISCUSSION

GENERAL DISCUSSION

Proteomic approaches have enabled the discovery of several potential biomarkers in biofluids. More recently focus has been given to saliva proteome analysis in oral and systemic diseases (Spielmann *et al.* 2011; Al Kawas *et al.* 2012; Amado *et al.* 2012; Liu *et al.* 2012). Nevertheless, little emphasis has been given to salivary proteome alterations induced by type 1 DM, and even less to salivary peptidome. For instance, Hirtz *et al.* (2006) evaluated the salivary proteome of poorly controlled type 1 DM patients and identified 23 proteins differentially expressed. Most of these proteins were underexpressed like isoforms of alpha-amylase, acidic PRPs, cystatin SA-1 and prolactin inducible protein. Two isoforms of serotransferrin, implicated in oral anti-inflammatory process were overexpressed, suggesting a disease-induced decrease of non-immunological defense in oral cavity. Cabras *et al.* (2010) highlighted the impairment of salivary peptides involved in the safeguard of the oral cavity in children with type 1 DM. These patients showed significant lower levels of statherin, SMR3B, aPRP103-169 and histatins and higher concentration of alpha-defensins 1, 2 and 4, S100A9. An additional important finding was the higher levels of aPRP103-169 fragments. These results could be explained by an increased salivary proteolytic activity in type 1 DM oral environment. Proteolysis plays a central role in pathophysiological events, being the proteases mainly involved in inflammatory processes and host defenses (Korkmaz *et al.* 2012). The increased serum levels of the zinc endopeptidases, particularly of MMP-2 and MMP-9, were already suggested as a marker of chronic kidney disease's risk (Gharagozlian *et al.* 2009) and of active retinopathy (Golubnitschaja *et al.* 2005).

In order to disclose the proteases involved in type 1 DM pathogenesis, we performed a straightforward profiling in the biofluids saliva, serum and urine from patients with no complications diagnosed, with nephropathy and with retinopathy using zymography-LC-MS/MS (Study II). From this characterization, the most outstanding disease-related proteolytic alterations were detected in urine and saliva. Serum exhibited less zymo bands in comparison to the other fluids and among the detected bands only the assigned by western blotting as MMP-2 and MMP-9 presented significant higher levels in patients with retinopathy and nephropathy, as previously reported (Thrailkill *et al.* 2007; Gharagozlian *et al.* 2009). In saliva and urine more zymo bands were observed and a higher number of proteases were identified and related with immune functions and biological events such as cell proliferation, secretion, invasion and angiogenesis. More distinct proteases were identified in urine than in saliva. Among the identified proteases modulated by DM

identified in urine samples were MMP-8, MMP-9, pro-MMP-9 NGAL/MMP-9, aminopeptidase N (APN), complement C1r subcomponent-like protein, kallikrein-1 and azurocidin. APN is a multifunctional enzyme related with immune system (Luan *et al.* 2007) and the significantly higher activity levels of this protease observed in diabetics with retinopathy and nephropathy, corroborating the results previously reported by Mitic *et al.* (2008) that observed a significantly higher activity of this protease in the urine from type 1 diabetic patients with microalbuminuria. Complement C1r subcomponent-like protein is a serine-type endopeptidase, highly expressed in kidney, but its physiological function remains to be determined. This protease may provide a novel means for the formation of the classical pathway C3/C5 convertase (Ligoudistianou *et al.* 2005). Kallikrein-1 and azurocidin were both identified in urine and saliva, with higher activity levels observed in DM patients with retinopathy and/or nephropathy. Azurocidin, a neutrophil granule-derived antibacterial and monocyte- and fibroblast-specific chemotactic glycoprotein was previously described as a promising biomarker in gingival crevicular fluid for the development of early diagnosis of periodontitis (Choi *et al.* 2011).

Disease-related increase activity and expression of MMPs was also observed in saliva and urine of type 1 DM patients. MMPs are involved in extracellular matrix vascular remodeling and their higher activity in the urine was previously suggested to predict vascular remodeling in type 1 DM renal and other vascular complications (McKittrick *et al.* 2011). More than MMP-9 *per se*, the complex involving this protease and neutrophil gelatinase-associated lipocalin (NGAL/MMP-9) was also proposed by Kim *et al.* (2012) as an earlier marker of nephropathy. In our study, NGAL/MMP-9 complex did not appear as a specific marker of nephropathy but instead of type 1 diabetes since it was present in higher levels in the urine of diabetics with and without complications. The overexpression of MMP-2 and MMP-8 was only detected in the saliva of diabetics, with significant higher levels of MMP-2 only noticed in patients with retinopathy and nephropathy. Increased levels of salivary MMP-8 were previously associated with type 2 DM-related advanced periodontitis (Collin *et al.* 2000).

Although present in low amounts, MMPs can produce a high number of protein fragments, modifying not only proteins but also their products yielding a characteristic peptide signature. In order to identify a potential association between type 1 DM-related higher proteolysis and peptidome profile, LC-MS/MS analysis of saliva was performed (Study I). Peptidome profiling of this biofluid evidenced several fragments of type I collagen as a result of disease-related increased proteolytic activity. Despite of the high intra- and inter-individual qualitative and quantitative diversity of the salivary peptidome (Le Yondre *et al.*

2008; Quintana *et al.* 2009; Vitorino *et al.* 2009), diabetic subjects showed an overall higher amount of protein fragments corroborated by higher intensity bands in zymography analysis. It was possible to identify MMP-9 and cathepsin D in zymo band with higher activity. Nevertheless, one may not forget the contribution of other proteases, as identified in study II, to the overall increased proteolytic activity noticed in diabetics. The observation of a consistent salivary protein degradation pattern in DM patients can pave the way for exploiting this feature for diagnosis purposes, being seen as an "fingerprint" of the pathophysiological status (Helmerhorst 2007; Amado *et al.* 2012). Serum peptidome analysis already allowed distinguishing metastatic thyroid carcinoma from cancer-free controls based only on its profile (Villanueva *et al.* 2008). Several saliva peptidome profiling approaches have been applied to pathologies like autism, caries and other oral diseases, HNC and diabetes (Castagnola *et al.* 2008; Huang *et al.* 2009; Cabras *et al.* 2010; Vitorino *et al.* 2010; Zhang *et al.* 2012). In study I, we not only characterized type 1 DM salivary peptidome but also the major protein cleavage sites were established in order to investigate potential proteases responsible for oral health alterations. Regarding the cleavage site frequencies, the motifs Phe-Tyr in salivary gland secreted peptides (SGP), Gly-Ala, Gln-Pro and Glu-Pro in nonsalivary gland secreted peptides (NSGP) were the more prevalent ones in diabetics. The increased cleavage observed in Phe-Tyr motif may be justified by disease-related increase of MMP-9 activity observed in both studies I and II or, according to MEROPS (Rawlings *et al.* 2008) to MMP-3, cathepsin G and cathepsin L, well described in human saliva (Pederson *et al.* 1995; Sun *et al.* 2009; Brik *et al.* 2010) and to several bacterial proteases. Concerning the Gly-Ala motif, the activity of MMP-2 and MMP-9 observed in study II might again justify the observed cleavage increase in type 1 DM patients along with other candidates such as MMP-3, MMP-12, MMP-13, cathepsin L and dipeptidyl-dipeptidase 1 and 2, already reported in saliva (Sun *et al.* 2009).

Many peptides from the extracellular matrix and cellular structural components were also found in diabetics' saliva samples, which may be associated with the infiltration of inflammatory cells and with the less collagen observed in the histological analysis of gingival biopsies from diabetic patients (Kumar *et al.* 2006; Lorencini *et al.* 2009). A higher number of fragments from type I collagen was observed in diabetic patients, which may be associated with a chronic inflammatory state, and with an inherent increasing risk of developing oral pathologies, namely gingivitis and periodontitis (Edwards *et al.* 2008; Orbak *et al.* 2008; Tanwir *et al.* 2009). MMPs are capable of degrading extracellular matrix components and to modulate immune response by altering activity of cytokines and

chemokines by cleavage (Silva *et al.* 2008; Negrato *et al.* 2010). Moreover, Salvi *et al.* (2010) suggested that elevated levels of IL-1 in type 1 DM patients regulates collagen metabolism, mainly through MMPs expression. In particular, MMP-8 and MMP-9 identified in study II, produced by polymorphonuclear leukocytes and osteoclasts, have been associated to periodontitis and other oral infections, being related with the increase of collagenolytic fragments (Kinney *et al.* 2007; Bildt *et al.* 2008; Gursoy *et al.* 2010). Data from study I is also in accordance with the significantly higher expression of MMP-9 observed in study III and with previous studies that reported increased salivary levels of this MMP in DM-related chronic periodontitis (Kaplan *et al.* 1978; Kumar *et al.* 2006).

While the biomarkers for DM diagnosis are well established (Assoc 2012) providing a good foundation for disease diagnosis, the available indicators that enable to predict the development of DM and its related chronic complications are clearly unsatisfactory (Padrao *et al.* 2012). So, the identification of DM-related biomarkers, especially targeted to the early diagnosis of DM chronic complications would be valuable for the prevention of disease occurrence and progression (Kuzuya *et al.* 2002). For the screen of non-physiological levels of certain proteins and/or peptides that may reflect pathological conditions quantitative proteomics has been increasingly recognized (Castagnola *et al.* 2011). With this in mind, study III was performed to determine salivary proteome and peptidome profiles among diabetics (with and without chronic complications) and healthy individuals using an iTRAQ-based experimental approach.

From the comparative analysis of saliva proteome, several proteins were identified as potential markers to distinguish individuals with type 1 DM as well as the ones with chronic complications associated with this disease. The majority of differentially expressed proteins are involved in metabolic (23.3 %) and immune response (23.3 %) processes, which corroborates the nature of the pathology, e.g. a metabolic disorder accompanied with inflammation (Llaurado *et al.* 2012). The significant upregulation of bactericidal/permeability-increasing protein-like 1 (BPI) and pancreatic adenocarcinoma factor (PAUF) in the saliva of diabetics suggests the activation of the immune system in type 1 DM. Moreover, the DM-related inflammatory response seems more exacerbated in patients with microvascular complications considering the high salivary levels of alpha-2-macroglobulin, defensin alpha 3 neutrophil-specific, leukocyte elastase inhibitor, matrix metalloproteinase-9 and neutrophil elastase. Other molecular players of the inflammatory process like actin bundling protein L-plastin, S100-A8 and S100-A9 were found in higher content in the saliva of diabetics, particularly in the ones with chronic complications. The upregulation of L-plastin was previously described in subjects with type 2 DM (Chan *et al.*

2012). The higher content of S100-A8 and S100-A9 observed in diabetics suggests that in these patients occurs the formation of the heterodimer known as Myeloid-Related Protein-8/14 (MRP8/14), which binds to the receptor for advanced glycation end products and Toll-like receptor 4, mediating intracellular inflammatory signaling transduction (Schafer *et al.* 1996; Croce *et al.* 2009; Xu *et al.* 2012). Increased levels of MRP8/14 complex in plasma were suggested as a prediction factor for increased risk of cardiovascular events (Morrow *et al.* 2008) and associated with inflammatory diseases, especially rheumatoid arthritis (Hammer *et al.* 2010). Data from study III suggest an association between increased salivary content of MRP8/14 and microvascular complications, which were not described before and could be used in the future in the monitoring of DM-related chronic complications.

Salivary peptidome profiling was also performed in study III aiming to add new insights on the effect of type 1 DM (Study I) and related chronic complications (Study III) on oral cavity homeostasis and consequent physiological impairment. A straightforward analysis of low molecular weight salivary peptides was performed using an iTRAQ experiment for quantitative comparison of peptidome profiles. Overall, a higher content of peptide fragments from the main salivary protein classes, mainly from aPRP, bPRP1 and bPRP2, was observed in subjects with diabetes, which seems to be related with the higher proteolytic activity reported in saliva, namely of MMP-9 (Studies I and II). This data was also corroborated by MEROPS search (Rawlings *et al.* 2012), considering the more prevalent cleavage sites. The analysis of the unique peptides in each study group evidenced an enrichment of N-terminal peptides from bPRP1 and C-terminal peptides from aPRP in diabetics. The elevated content of aPRP peptide fragments (P-C) was previously reported in type 1 diabetic childrens (Cabras *et al.* 2010). Although bPRP's role in oral cavity is not well characterized, in the case of aPRP, the C-terminal peptide (^{148}GPPPQGGRPQGPPQGQSPQ^{166}) is known to be associated with bacterial attachment to tooth surface, avoiding bacterial binding (Gibbons *et al.* 1991). The elevated levels of both aPRP C-terminal peptide and BPI observed in the saliva of diabetics might be seen as a protective mechanism against bacterial infection that predisposes to tooth demineralization (Huang *et al.* 2008; Levine 2011).

In order to get a deeper perspective of the salivary peptidome dynamics, other post-translation modifications besides proteolysis were analyzed. Data obtained points to a functional impact of phosphorylation and glycosylation, being detected a higher number of phosphopeptides and HexNAc modification in bPRPs in diabetics. Regarding the most prevalent phosphopeptides, no significant expression differences were detected. In fact,

phosphorylation of PRPs, sthaterin and histatin 1 has been associated with the acquired enamel pellicle formation and, consequently, with the participation in the remineralization process, having a protective effect against tooth erosion (Vitorino *et al.* 2008; Siqueira *et al.* 2009). In respect to the higher levels of HexNac modification those might reflect the O-GlcNAcylation observed in diabetes (Ngoh *et al.* 2010).

In order to integrate all data retrieved from the three experimental studies, mainly focused on saliva proteome and peptidome alterations induced by type 1 DM and related complications, a protein network was constructed with STRING (Szklarczyk *et al.* 2011). Figure 1 illustrates protein-protein interactions, upon querying the database with the participating 77 identified proteins (Studies I-III). Three clusters (presented with distinct colors in figure 1) are evidenced and are tightly connected to the functional modules: one cluster comprehending collagen type II, collagen type I subunits interaction and MMP-9; a second cluster involving MMP-2 and collagen type I; and a third cluster comprehending salivary and inflammatory proteins. According to Kegg pathways, these clusters are mainly associated to extracellular matrix-receptor interaction, focal adhesion, pathways in cancer and leukocyte transendothelial migration.

Moreover, data from studies I-III corroborate the potential of saliva for biomarker discovery in type 1 DM and related complications. The salivary proteome and even more the peptidome have been quite unexplored in pathophysiological conditions. The straightforward approach applied in our study highlights the potential of evaluating peptide levels and PTMs to identify new peptide targets for type 1 DM diagnosis/monitoring.

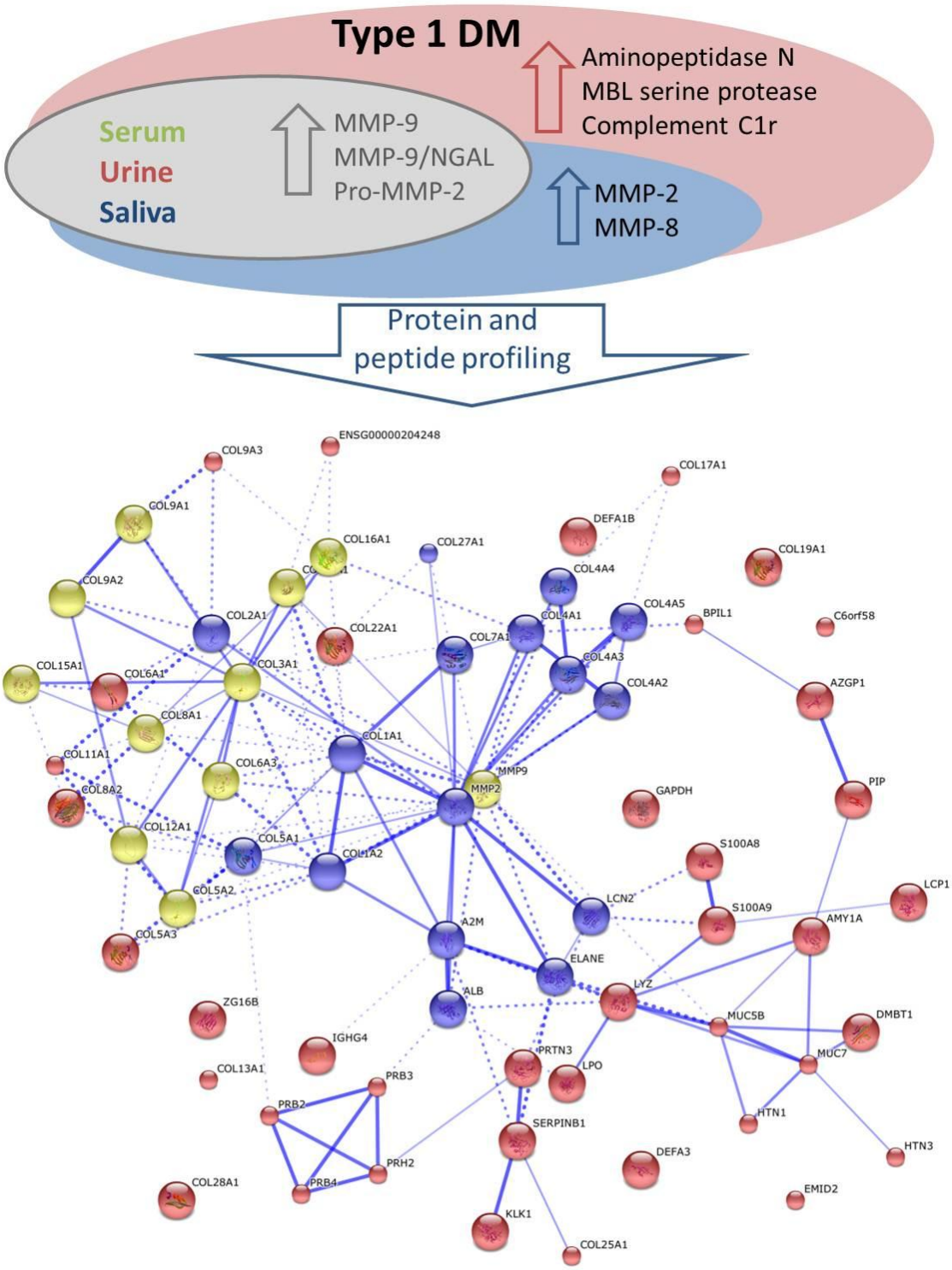

Figure 1: STRING protein network that integrate all data retrieved from the three experimental studies and shows protein-protein interactions, evidencing three clusters: one cluster comprehending collagen type II, collagen type I subunits interaction and MMP-9 (blue nodes); a second cluster involving MMP-2 and collagen type I (yellow nodes); and a third cluster comprehending salivary and inflammatory proteins (red nodes).

CHAPTER IV

CONCLUSIONS

CONCLUSIONS

Once characterized the alterations in salivary proteome and peptidome induced by type 1 DM and related microvascular complications, the following conclusions might be envisioned:

i) Type 1 DM and particularly the associated microvascular complications modulates biofluids' proteolytic profile, with significant activity differences noticed for urine and saliva with MMP-9/Neutrophil gelatinase-associated lipocalin, aminopeptidase N, azurocidin and kallikrein 1 as promisors disease-related screening targets;

ii) The higher proteolytic activity noticed in whole saliva of diabetics leads to an increase in the percentage of peptides, mainly consisting of an augmented number of collagen type I fragments, possibly reflecting a chronic inflammatory state of oral and periodontal tissues;

iii) Peptidome data support a diabetes-related higher susceptibility of salivary proteins to proteolysis and evidenced an increased content of some specific protein fragments associated with bacterial attachment and the accumulation of phosphopeptides potentially involved in tooth protection; moreover, proteolytic fragments from bPRP1, bPRP2 and aPRP might be seen as a hallmark of the disease pathogenesis with potential use for its monitoring;

iv) Salivary proteome profiling evidenced an overexpression of L-plastin, PAUF and MRP8/14, endogenous ligands of TLR2 and TLR4, proteins known to be involved in inflammatory processes, in the saliva of subjects with diabetes-related retinopathy and nephropathy.

Overall, the experimental studies presented in this thesis emphasize the importance of the innate immune system in the pathogenesis of type 1 DM and related microvascular complications and the relevance of proteolytic events that lead to an increased content of endogenous peptides many with putative disease biomarker potential. An integrated perspective of salivary proteome and peptidome is highlighted and open new avenues for future studies focused in the evaluation of the early predictive value of the potential biomarkers suggested for the diagnosis and prognosis of type 1 DM and related complications. For the translation of those potential targets to clinics, a deeper high-throughput analysis, in a large scale population, is required. Moreover, disclose the functional role of the identified salivary peptides will contribute to a deeper knowledge in pathophysiology of DM-related oral complications.

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APENDIX -
SUPPLEMENTARY DATA

STUDY I - SALIVARY PEPTIDOME IN TYPE 1 DIABETES MELLITUS

Supplemental Table 1: List of proteins that correspond to the identified peptides in all subjects.

Protein Name	Accession Number	Protein MW	Protein PI
Acetylcholinesterase collagenic tail peptide OS=Homo sapiens GN=COLQ PE=1 SV=2	COLQ_HUMAN	47735,3086	8,42
Atherin OS=Homo sapiens GN=SAMD1 PE=1 SV=1	SAMD1_HUMAN	56017,7070	7,12
AT-rich interactive domain-containing protein 1B OS=Homo sapiens GN=ARID1B PE=1 SV=2	ARI1B_HUMAN	235973,8594	6,26
Basic proline-rich peptide P-E OS=Homo sapiens PE=1 SV=1	PRPE_HUMAN	6020,0801	11,74
Basic salivary proline-rich protein 1 OS=Homo sapiens GN=PRB1 PE=1 SV=2	PRP1_HUMAN	38522,6211	11,22
Basic salivary proline-rich protein 2 OS=Homo sapiens GN=PRB2 PE=1 SV=3	PRB2_HUMAN	40774,7695	11,63
Basic salivary proline-rich protein 3 OS=Homo sapiens GN=PRB3 PE=1 SV=1	PRB3_HUMAN	30917,5605	10,61
Basic salivary proline-rich protein 4 OS=Homo sapiens GN=PRB4 PE=1 SV=3	PRB4_HUMAN	31332,7793	10,52
BAT2 domain-containing protein 1 OS=Homo sapiens GN=BAT2D1 PE=1 SV=2	BA2D1_HUMAN	316843,6250	9,17
Choline kinase alpha OS=Homo sapiens GN=CHKA PE=1 SV=2	CHKA_HUMAN	52168,5313	6,16
Cleavage and polyadenylation specificity factor subunit 6 OS=Homo sapiens GN=CPSF6 PE=1 SV=2	CPSF6_HUMAN	59173,4219	6,66
Collagen alpha-1(I) chain OS=Homo sapiens GN=COL1A1 PE=1 SV=4	CO1A1_HUMAN	138826,5469	5,60
Collagen alpha-1(II) chain OS=Homo sapiens GN=COL2A1 PE=1 SV=3	CO2A1_HUMAN	141698,5000	6,58
Collagen alpha-1(III) chain OS=Homo sapiens GN=COL3A1 PE=1 SV=4	CO3A1_HUMAN	138478,9688	6,21
Collagen alpha-1(IV) chain OS=Homo sapiens GN=COL4A1 PE=1 SV=3	CO4A1_HUMAN	160514,0469	8,55
Collagen alpha-1(IX) chain OS=Homo sapiens GN=COL9A1 PE=1 SV=2	CO9A1_HUMAN	91798,4922	8,93
Collagen alpha-1(V) chain OS=Homo sapiens GN=COL5A1 PE=1 SV=3	CO5A1_HUMAN	183447,0469	4,94
Collagen alpha-1(VI) chain OS=Homo sapiens GN=COL6A1 PE=1 SV=3	CO6A1_HUMAN	108461,9844	5,26
Collagen alpha-1(VII) chain OS=Homo sapiens GN=COL7A1 PE=1 SV=2	CO7A1_HUMAN	295040,9375	5,95
Collagen alpha-1(VIII) chain OS=Homo sapiens GN=COL8A1 PE=2 SV=2	CO8A1_HUMAN	73317,1875	9,62
Collagen alpha-1(X) chain OS=Homo sapiens GN=COL10A1 PE=1 SV=2	COAA1_HUMAN	66116,7969	9,68
Collagen alpha-1(XI) chain OS=Homo sapiens GN=COL11A1 PE=1 SV=4	COBA1_HUMAN	180953,0000	5,06

Collagen alpha-1(XII) chain OS=Homo sapiens GN=COL12A1 PE=1 SV=2	COCA1_HUMAN	332939,5938	5,38
Collagen alpha-1(XIII) chain OS=Homo sapiens GN=COL13A1 PE=1 SV=1	CODA1_HUMAN	69906,6172	9,27
Collagen alpha-1(XIX) chain OS=Homo sapiens GN=COL19A1 PE=1 SV=3	COJA1_HUMAN	115149,3906	8,57
Collagen alpha-1(XV) chain OS=Homo sapiens GN=COL15A1 PE=1 SV=2	COFA1_HUMAN	141631,7031	4,89
Collagen alpha-1(XVI) chain OS=Homo sapiens GN=COL16A1 PE=1 SV=2	COGA1_HUMAN	157652,5469	8,14
Collagen alpha-1(XVII) chain OS=Homo sapiens GN=COL17A1 PE=1 SV=3	COHA1_HUMAN	150326,4844	8,89
Collagen alpha-1(XXII) chain OS=Homo sapiens GN=COL22A1 PE=1 SV=1	COMA1_HUMAN	161015,5469	6,88
Collagen alpha-1(XXV) chain OS=Homo sapiens GN=COL25A1 PE=1 SV=1	COPA1_HUMAN	64745,5117	8,80
Collagen alpha-1(XXVI) chain OS=Homo sapiens GN=EMID2 PE=2 SV=1	EMID2_HUMAN	45352,6211	7,02
Collagen alpha-1(XXVII) chain OS=Homo sapiens GN=COL27A1 PE=1 SV=1	CORA1_HUMAN	186776,0938	9,83
Collagen alpha-1(XXVII) chain OS=Homo sapiens GN=COL27A1 PE=1 SV=1	CORA1_HUMAN	186776,0938	9,83
Collagen alpha-1(XXVIII) chain OS=Homo sapiens GN=COL28A1 PE=2 SV=2	COSA1_HUMAN	116584,8594	6,10
Collagen alpha-2(I) chain OS=Homo sapiens GN=COL1A2 PE=1 SV=6	CO1A2_HUMAN	129209,4219	9,08
Collagen alpha-2(IV) chain OS=Homo sapiens GN=COL4A2 PE=1 SV=4	CO4A2_HUMAN	167448,6250	8,89
Collagen alpha-2(IX) chain OS=Homo sapiens GN=COL9A2 PE=1 SV=2	CO9A2_HUMAN	65090,9297	9,23
Collagen alpha-2(IX) chain OS=Homo sapiens GN=COL9A2 PE=1 SV=2	CO9A2_HUMAN	65090,9297	9,23
Collagen alpha-2(V) chain OS=Homo sapiens GN=COL5A2 PE=1 SV=3	CO5A2_HUMAN	144820,9688	6,07
Collagen alpha-2(VIII) chain OS=Homo sapiens GN=COL8A2 PE=1 SV=2	CO8A2_HUMAN	67202,3438	9,05
Collagen alpha-2(XI) chain OS=Homo sapiens GN=COL11A2 PE=1 SV=4	COBA2_HUMAN	171669,8438	5,89
Collagen alpha-3(IV) chain OS=Homo sapiens GN=COL4A3 PE=1 SV=3	CO4A3_HUMAN	161711,0625	9,28
Collagen alpha-3(IX) chain OS=Homo sapiens GN=COL9A3 PE=2 SV=2	CO9A3_HUMAN	63577,1211	7,57
Collagen alpha-3(V) chain OS=Homo sapiens GN=COL5A3 PE=1 SV=2	CO5A3_HUMAN	171946,7031	6,37
Collagen alpha-3(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=4	CO6A3_HUMAN	343452,7813	6,26
Collagen alpha-4(IV) chain OS=Homo sapiens GN=COL4A4 PE=1 SV=2	CO4A4_HUMAN	163895,3125	8,87
Collagen alpha-5(IV) chain OS=Homo sapiens GN=COL4A5 PE=1 SV=2	CO4A5_HUMAN	160942,9063	7,71
Collagen alpha-6(IV) chain OS=Homo sapiens GN=COL4A6 PE=2 SV=3	CO4A6_HUMAN	163704,2813	9,31
Collectin-12 OS=Homo sapiens GN=COLEC12 PE=1 SV=2	COL12_HUMAN	81474,6797	5,48
Complement C1q tumor necrosis factor-related protein 2 OS=Homo sapiens GN=C1QTNF2 PE=2 SV=1	C1QT2_HUMAN	29932,8809	9,04
Constitutive coactivator of PPAR-gamma-like protein 2 OS=Homo sapiens GN=FAM120C PE=2 SV=2	F120C_HUMAN	120499,5469	9,19
Corticotropin-lipotropin OS=Homo sapiens GN=POMC PE=1 SV=2	COLI_HUMAN	29405,3691	7,56

Dedicator of cytokinesis protein 5 OS=Homo sapiens GN=DOCK5 PE=1 SV=3	DOCK5_HUMAN	215172,0469	8,08
Delphilin OS=Homo sapiens GN=GRID2IP PE=2 SV=2	GRD2I_HUMAN	132193,7031	7,11
Disheveled-associated activator of morphogenesis 2 OS=Homo sapiens GN=DAAM2 PE=2 SV=3	DAAM2_HUMAN	123420,3594	6,36
Double C2-like domain-containing protein alpha OS=Homo sapiens GN=DOC2A PE=1 SV=5	DOC2A_HUMAN	43931,4727	6,84
Dynamin-2 OS=Homo sapiens GN=DNM2 PE=1 SV=2	DYN2_HUMAN	98003,1563	7,04
E1A-binding protein p400 OS=Homo sapiens GN=EP400 PE=1 SV=3	EP400_HUMAN	343403,6875	9,27
E3 ubiquitin-protein ligase CBL-B OS=Homo sapiens GN=CBLB PE=1 SV=2	CBLB_HUMAN	109380,4531	8,15
Elastin OS=Homo sapiens GN=ELN PE=1 SV=2	ELN_HUMAN	68456,6484	10,40
EMILIN-1 OS=Homo sapiens GN=EMILIN1 PE=1 SV=2	EMIL1_HUMAN	106601,4531	5,07
Enolase-like protein C10orf134 OS=Homo sapiens GN=C10orf134 PE=2 SV=2	ENOLL_HUMAN	68777,3125	5,70
Espin OS=Homo sapiens GN=ESPN PE=1 SV=1	ESPN_HUMAN	91675,2344	6,47
Far upstream element-binding protein 2 OS=Homo sapiens GN=KHSRP PE=1 SV=3	FUBP2_HUMAN	73101,1094	6,84
Fas-activated serine/threonine kinase OS=Homo sapiens GN=FASTK PE=1 SV=1	FASTK_HUMAN	61065,8359	9,96
Fibrillin-2 OS=Homo sapiens GN=FBN2 PE=1 SV=2	FBN2_HUMAN	314130,6563	4,73
Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2	FIBB_HUMAN	55892,2617	8,54
Forkhead box protein N1 OS=Homo sapiens GN=FOXN1 PE=2 SV=1	FOXN1_HUMAN	68881,1797	5,93
Galectin-3 OS=Homo sapiens GN=LGALS3 PE=1 SV=5	LEG3_HUMAN	26136,0508	8,57
Glutamate [NMDA] receptor subunit epsilon-4 OS=Homo sapiens GN=GRIN2D PE=1 SV=1	NMDE4_HUMAN	143468,8594	8,55
Glutamate [NMDA] receptor subunit epsilon-4 OS=Homo sapiens GN=GRIN2D PE=1 SV=2	NMDE4_HUMAN	143661,0938	8,68
GON-4-like protein OS=Homo sapiens GN=GON4L PE=1 SV=1	GON4L_HUMAN	248465,3438	4,94
Helicase SRCAP OS=Homo sapiens GN=SRCAP PE=1 SV=2	SRCAP_HUMAN	343312,8125	5,69
Heterogeneous nuclear ribonucleoprotein U-like protein 1 OS=Homo sapiens GN=HNRNPUL1 PE=1 SV=2	HNRL1_HUMAN	95679,2500	6,49
Histatin-1 OS=Homo sapiens GN=HTN1 PE=1 SV=2	HIS1_HUMAN	6958,3799	9,11
Histatin-3 OS=Homo sapiens GN=HTN3 PE=1 SV=2	HIS3_HUMAN	6145,0898	10,09
Histone acetyltransferase MYST3 OS=Homo sapiens GN=MYST3 PE=1 SV=2	MYST3_HUMAN	224884,7813	5,50
Histone demethylase JMJD3 OS=Homo sapiens GN=JMJD3 PE=1 SV=3	JMJD3_HUMAN	180298,5156	9,01
Histone-lysine N-methyltransferase MLL2 OS=Homo sapiens GN=MLL2 PE=1 SV=1	MLL2_HUMAN	563830,9375	5,65
Histone-lysine N-methyltransferase MLL4 OS=Homo sapiens GN=WBP7 PE=1 SV=1	MLL4_HUMAN	293327,3125	8,59
Histone-lysine N-methyltransferase MLL5 OS=Homo sapiens GN=MLL5 PE=1 SV=1	MLL5_HUMAN	204836,6094	7,41
Histone-lysine N-methyltransferase NSD3 OS=Homo sapiens GN=WHSC1L1 PE=1 SV=1	NSD3_HUMAN	161509,0156	8,57

Histone-lysine N-methyltransferase SETD1A OS=Homo sapiens GN=SETD1A PE=1 SV=3	SET1A_HUMAN	185919,1406	5,07
Histone-lysine N-methyltransferase SETD1B OS=Homo sapiens GN=SETD1B PE=1 SV=2	SET1B_HUMAN	208599,4219	4,86
Homeobox protein CDX-2 OS=Homo sapiens GN=CDX2 PE=1 SV=2	CDX2_HUMAN	33488,9453	9,65
Homeobox protein Hox-A10 OS=Homo sapiens GN=HOXA10 PE=1 SV=2	HXA10_HUMAN	40510,7305	8,30
Host cell factor OS=Homo sapiens GN=HCFC1 PE=1 SV=2	HCFC1_HUMAN	208600,9688	7,32
Huntingtin OS=Homo sapiens GN=HTT PE=1 SV=1	HD_HUMAN	347638,8750	5,81
Insulinoma-associated protein 1 OS=Homo sapiens GN=INSM1 PE=2 SV=1	INSM1_HUMAN	52890,0195	9,19
Inverted formin-2 OS=Homo sapiens GN=INF2 PE=1 SV=2	INF2_HUMAN	135539,7344	5,26
IQ motif and SEC7 domain-containing protein 3 OS=Homo sapiens GN=IQSEC3 PE=2 SV=3	IQEC3_HUMAN	127541,4531	6,08
Iroquois-class homeodomain protein IRX-1 OS=Homo sapiens GN=IRX1 PE=1 SV=3	IRX1_HUMAN	49590,8398	5,78
Kelch-like protein 17 OS=Homo sapiens GN=KLHL17 PE=2 SV=1	KLH17_HUMAN	69830,1250	7,64
Ladybird homeobox corepressor 1-like protein OS=Homo sapiens GN=CORL2 PE=1 SV=2	CORL2_HUMAN	104170,2578	6,02
Large proline-rich protein BAT2 OS=Homo sapiens GN=BAT2 PE=1 SV=2	BAT2_HUMAN	228720,5781	9,49
LON peptidase N-terminal domain and RING finger protein 2 OS=Homo sapiens GN=LONRF2 PE=2 SV=2	LONF2_HUMAN	83584,3281	5,65
MAP kinase-activated protein kinase 2 OS=Homo sapiens GN=MAPKAPK2 PE=1 SV=1	MAPK2_HUMAN	45538,4844	8,87
Matrix metalloproteinase-15 OS=Homo sapiens GN=MMP15 PE=1 SV=1	MMP15_HUMAN	75759,0469	7,03
Mediator of RNA polymerase II transcription subunit 25 OS=Homo sapiens GN=MED25 PE=1 SV=2	MED25_HUMAN	78121,4219	8,61
Methyl-CpG-binding domain protein 6 OS=Homo sapiens GN=MBD6 PE=2 SV=2	MBD6_HUMAN	101138,4609	9,72
Mucin-7 OS=Homo sapiens GN=MUC7 PE=1 SV=1	MUC7_HUMAN	39146,6992	8,99
Multivesicular body subunit 12B OS=Homo sapiens GN=FAM125B PE=1 SV=2	F125B_HUMAN	35596,8359	8,42
Myocyte-specific enhancer factor 2A OS=Homo sapiens GN=MEF2A PE=1 SV=1	MEF2A_HUMAN	54777,1602	7,72
Myosin-Ie OS=Homo sapiens GN=MYO1E PE=1 SV=2	MYO1E_HUMAN	126981,9922	9,01
Neurogenic differentiation factor 2 OS=Homo sapiens GN=NEUROD2 PE=2 SV=2	NDF2_HUMAN	41335,3203	6,29
Neurogenic locus notch homolog protein 1 OS=Homo sapiens GN=NOTCH1 PE=1 SV=4	NOTC1_HUMAN	272320,7188	4,95
Neurogenic locus notch homolog protein 3 OS=Homo sapiens GN=NOTCH3 PE=1 SV=1	NOTC3_HUMAN	243496,1563	5,18
Peripheral-type benzodiazepine receptor-associated protein 1 OS=Homo sapiens GN=BZRAP1 PE=1 SV=2	RIMB1_HUMAN	199927,6719	5,05
PERQ amino acid-rich with GYF domain-containing protein 1 OS=Homo sapiens GN=GIGYF1 PE=1 SV=2	PERQ1_HUMAN	114531,1875	5,29
Pleckstrin homology domain-containing family G member 2 OS=Homo sapiens GN=PLEKHG2 PE=1 SV=2	PKHG2_HUMAN	147823,0469	5,57
Pogo transposable element with ZNF domain OS=Homo sapiens GN=POGZ PE=1 SV=2	POGZ_HUMAN	155244,6875	7,14
Polymeric immunoglobulin receptor OS=Homo sapiens GN=PIGR PE=1 SV=4	PIGR_HUMAN	83231,6484	5,58

Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2 OS=Homo sapiens GN=H	HCN2_HUMAN	96888,3438	9,15
POU domain, class 3, transcription factor 3 OS=Homo sapiens GN=POU3F3 PE=2 SV=2	PO3F3_HUMAN	50295,7383	7,51
Pre-mRNA 3'-end-processing factor FIP1 OS=Homo sapiens GN=FIP1L1 PE=1 SV=1	FIP1_HUMAN	66486,6172	5,42
Probable G-protein coupled receptor 62 OS=Homo sapiens GN=GPR62 PE=2 SV=1	GPR62_HUMAN	37604,7656	10,99
Probable RNA-binding protein 20 OS=Homo sapiens GN=RBM20 PE=2 SV=2	RBM20_HUMAN	122387,5547	5,56
Programmed cell death protein 7 OS=Homo sapiens GN=PDCD7 PE=1 SV=1	PDCD7_HUMAN	54666,2109	9,97
Proline-, glutamic acid- and leucine-rich protein 1 OS=Homo sapiens GN=PELP1 PE=1 SV=2	PELP1_HUMAN	119624,5391	4,29
Proline-rich protein 12 OS=Homo sapiens GN=PRR12 PE=1 SV=2	PRR12_HUMAN	129910,7813	8,18
Proline-rich protein 4 OS=Homo sapiens GN=PRR4 PE=1 SV=2	PROL4_HUMAN	15115,6396	6,97
Proline-rich protein 6 OS=Homo sapiens GN=PRR6 PE=1 SV=1	PRR6_HUMAN	29927,1094	9,78
Protein bassoon OS=Homo sapiens GN=BSN PE=1 SV=3	BSN_HUMAN	416244,1563	7,28
Protein CBFA2T3 OS=Homo sapiens GN=CBFA2T3 PE=1 SV=2	MTG16_HUMAN	71147,6484	8,50
Protein cordon-bleu OS=Homo sapiens GN=COBL PE=1 SV=2	COBL_HUMAN	135533,4844	7,66
Protein deltex-1 OS=Homo sapiens GN=DTX1 PE=1 SV=1	DTX1_HUMAN	67325,4922	9,67
Protein FAM132B OS=Homo sapiens GN=FAM132B PE=2 SV=2	F132B_HUMAN	37255,6875	9,88
Protein FAM44A OS=Homo sapiens GN=FAM44A PE=1 SV=2	FA44A_HUMAN	330263,8438	5,00
Protein fosB OS=Homo sapiens GN=FOSB PE=1 SV=1	FOSB_HUMAN	35905,6133	4,78
Protein phosphatase PTC7 homolog OS=Homo sapiens GN=PPTC7 PE=2 SV=1	PPTC7_HUMAN	32625,1406	4,99
Protein TANC2 OS=Homo sapiens GN=TANC2 PE=1 SV=3	TANC2_HUMAN	219510,5781	8,31
Protein transport protein Sec24D OS=Homo sapiens GN=SEC24D PE=2 SV=2	SC24D_HUMAN	112936,4766	6,91
Protein virilizer homolog OS=Homo sapiens GN=KIAA1429 PE=1 SV=2	VIR_HUMAN	201897,1406	4,90
Protocadherin-15 OS=Homo sapiens GN=PCDH15 PE=1 SV=2	PCD15_HUMAN	215934,0000	4,94
Psoriasis susceptibility 1 candidate gene 2 protein OS=Homo sapiens GN=PSORS1C2 PE=2 SV=1	PS1C2_HUMAN	15075,4102	4,48
Pulmonary surfactant-associated protein A2 OS=Homo sapiens GN=SFTPA2 PE=1 SV=1	SFTA2_HUMAN	26165,6191	5,07
Putative protein unc-93 homolog B1-like protein OS=Homo sapiens PE=5 SV=3	U93BL_HUMAN	27206,0996	5,36
Putative uncharacterized protein LOC439951 OS=Homo sapiens PE=5 SV=1	YJ017_HUMAN	22292,0293	12,41
Ran-binding protein 9 OS=Homo sapiens GN=RANBP9 PE=1 SV=1	RANB9_HUMAN	77798,0313	6,31
RanBP-type and C3HC4-type zinc finger-containing protein 1 OS=Homo sapiens GN=RBCK1 PE=1 SV=2	UB7I3_HUMAN	57533,8711	5,47
Ras and Rab interactor 2 OS=Homo sapiens GN=RIN2 PE=1 SV=1	RIN2_HUMAN	100099,4375	6,15
Receptor-type tyrosine-protein phosphatase delta OS=Homo sapiens GN=PTPRD PE=1 SV=2	PTPRD_HUMAN	214624,8125	6,14

Regulator of telomere elongation helicase 1 OS=Homo sapiens GN=RTEL1 PE=2 SV=1	RTEL1_HUMAN	152277,9219	8,68
Retinal homeobox protein Rx OS=Homo sapiens GN=RAX PE=1 SV=1	RX_HUMAN	36781,8789	9,11
Retrotransposon-derived protein PEG10 OS=Homo sapiens GN=PEG10 PE=1 SV=2	PEG10_HUMAN	80122,1172	5,94
Rho GTPase-activating protein 17 OS=Homo sapiens GN=ARHGAP17 PE=1 SV=1	RHG17_HUMAN	95377,5078	7,23
RNA-binding protein 27 OS=Homo sapiens GN=RBM27 PE=1 SV=2	RBM27_HUMAN	118644,7500	9,24
RNA-binding protein 33 OS=Homo sapiens GN=RBM33 PE=1 SV=3	RBM33_HUMAN	129905,7500	6,45
Salivary acidic proline-rich phosphoprotein 1/2 OS=Homo sapiens GN=PRH1 PE=1 SV=2	PRPC_HUMAN	17006,2793	4,63
Sal-like protein 2 OS=Homo sapiens GN=SALL2 PE=1 SV=3	SALL2_HUMAN	105332,9297	5,92
SCO-spondin OS=Homo sapiens GN=SSPO PE=2 SV=1	SSPO_HUMAN	547136,0000	5,66
Serine/threonine-protein kinase LMTK3 OS=Homo sapiens GN=LMTK3 PE=2 SV=2	LMTK3_HUMAN	153565,7656	4,77
Serine/threonine-protein kinase WNK2 OS=Homo sapiens GN=WNK2 PE=1 SV=4	WNK2_HUMAN	242522,5469	5,79
SH3 and multiple ankyrin repeat domains protein 3 OS=Homo sapiens GN=SHANK3 PE=1 SV=2	SHAN3_HUMAN	186181,4844	9,13
SH3 and PX domain-containing protein 2B OS=Homo sapiens GN=SH3PXD2B PE=1 SV=3	SPD2B_HUMAN	101516,1094	8,82
SH3 domain-binding protein 2 OS=Homo sapiens GN=SH3BP2 PE=1 SV=2	3BP2_HUMAN	62204,1289	7,67
SLIT-ROBO Rho GTPase-activating protein 1 OS=Homo sapiens GN=SRGAP1 PE=1 SV=1	SRGP1_HUMAN	124186,3203	6,36
Spermatogenesis-associated protein 5-like protein 1 OS=Homo sapiens GN=SPATA5L1 PE=2 SV=1	SPA5L_HUMAN	80631,9375	8,29
Splicing factor 3B subunit 4 OS=Homo sapiens GN=SF3B4 PE=1 SV=1	SF3B4_HUMAN	44357,1602	8,54
Splicing factor, arginine/serine-rich 15 OS=Homo sapiens GN=SFRS15 PE=1 SV=3	SFR15_HUMAN	125790,4063	9,58
Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2	SFPQ_HUMAN	76101,7266	9,45
Statherin OS=Homo sapiens GN=STATH PE=1 SV=2	STAT_HUMAN	7299,6499	7,98
Stress-induced-phosphoprotein 1 OS=Homo sapiens GN=STIP1 PE=1 SV=1	STIP1_HUMAN	62599,4648	6,40
Submaxillary gland androgen-regulated protein 3B OS=Homo sapiens GN=SMR3B PE=1 SV=2	SMR3B_HUMAN	8182,2598	9,63
Synapsin-1 OS=Homo sapiens GN=SYN1 PE=1 SV=3	SYN1_HUMAN	74065,7188	9,84
T-box transcription factor TBX1 OS=Homo sapiens GN=TBX1 PE=1 SV=1	TBX1_HUMAN	43105,3398	8,37
Titin OS=Homo sapiens GN=TTN PE=1 SV=2	TITIN_HUMAN	3813809,5000	6,01
Transcription factor 20 OS=Homo sapiens GN=TCF20 PE=1 SV=3	TCF20_HUMAN	211639,0156	9,16
Transcription initiation factor TFIID subunit 6 OS=Homo sapiens GN=TAF6 PE=1 SV=1	TAF6_HUMAN	72623,0547	8,83
Transcriptional adapter 3-like OS=Homo sapiens GN=TADA3L PE=1 SV=1	TAD3L_HUMAN	48872,2617	5,90
Transcriptional-regulating factor 1 OS=Homo sapiens GN=TRERF1 PE=1 SV=1	TREF1_HUMAN	132172,7500	6,26
Transforming acidic coiled-coil-containing protein 3 OS=Homo sapiens GN=TACC3 PE=1 SV=1	TACC3_HUMAN	90303,7500	4,97

Tyrosine-protein kinase SgK223 OS=Homo sapiens GN=SGK223 PE=1 SV=2	SG223_HUMAN	149593,1094	6,76
Uncharacterized protein C12orf34 OS=Homo sapiens GN=C12orf34 PE=2 SV=1	CL034_HUMAN	46762,0469	9,20
Uncharacterized protein C2orf78 OS=Homo sapiens GN=C2orf78 PE=2 SV=2	CB078_HUMAN	96939,7656	9,11
Uncharacterized protein C4orf40 OS=Homo sapiens GN=C4orf40 PE=1 SV=1	CD040_HUMAN	22705,5391	4,82
Uncharacterized protein C6orf132 OS=Homo sapiens GN=C6orf132 PE=2 SV=3	CF132_HUMAN	124016,3203	9,46
Uncharacterized protein C9orf40 OS=Homo sapiens GN=C9orf40 PE=1 SV=1	CI040_HUMAN	21050,3789	4,89
Uncharacterized protein KIAA1522 OS=Homo sapiens GN=KIAA1522 PE=1 SV=2	K1522_HUMAN	107030,1094	9,75
Utrophin OS=Homo sapiens GN=UTRN PE=1 SV=2	UTRO_HUMAN	394219,3438	5,20
Voltage-dependent T-type calcium channel subunit alpha-1I OS=Homo sapiens GN=CACNA1I PE=1 SV=1	CAC1I_HUMAN	244942,8281	6,09
WAS/WASL-interacting protein family member 2 OS=Homo sapiens GN=WIPF2 PE=1 SV=1	WIPF2_HUMAN	46260,1719	10,93
WAS/WASL-interacting protein family member 3 OS=Homo sapiens GN=WIPF3 PE=2 SV=3	WIPF3_HUMAN	49317,9492	10,01
WD repeat-containing protein 33 OS=Homo sapiens GN=WDR33 PE=1 SV=2	WDR33_HUMAN	145799,2031	9,24
Wiskott-Aldrich syndrome protein family member 2 OS=Homo sapiens GN=WASF2 PE=1 SV=3	WASF2_HUMAN	54250,5391	5,38
Wiskott-Aldrich syndrome protein family member 3 OS=Homo sapiens GN=WASF3 PE=2 SV=2	WASF3_HUMAN	55258,7734	6,00
Wiskott-Aldrich syndrome protein OS=Homo sapiens GN=WAS PE=1 SV=4	WASP_HUMAN	52879,8594	6,18
WW domain-binding protein 11 OS=Homo sapiens GN=WBP11 PE=1 SV=1	WBP11_HUMAN	69953,9219	8,28
Xin actin-binding repeat-containing protein 1 OS=Homo sapiens GN=XIRP1 PE=1 SV=1	XIRP1_HUMAN	198439,0000	5,78
YLP motif-containing protein 1 OS=Homo sapiens GN=YLPM1 PE=1 SV=3	YLPM1_HUMAN	219847,5625	6,14
Zinc finger and BTB domain-containing protein 20 OS=Homo sapiens GN=ZBTB20 PE=2 SV=3	ZBT20_HUMAN	81032,3906	6,03
Zinc finger CCHC domain-containing protein 2 OS=Homo sapiens GN=ZCCHC2 PE=1 SV=6	ZCHC2_HUMAN	125857,8516	6,55
Zinc finger homeobox protein 2 OS=Homo sapiens GN=ZFHX2 PE=2 SV=2	ZFHX2_HUMAN	152005,8125	6,00
Zinc finger homeobox protein 3 OS=Homo sapiens GN=ZFHX3 PE=1 SV=2	ZFHX3_HUMAN	404165,4688	5,82
Zinc finger MIZ domain-containing protein 1 OS=Homo sapiens GN=ZMIZ1 PE=1 SV=3	ZMIZ1_HUMAN	115408,8828	7,09
Zinc finger protein 341 OS=Homo sapiens GN=ZNF341 PE=2 SV=2	ZN341_HUMAN	92668,7500	9,11
Zinc finger protein 469 OS=Homo sapiens GN=ZNF469 PE=1 SV=3	ZN469_HUMAN	409949,0938	7,88
Zinc finger protein 827 OS=Homo sapiens GN=ZNF827 PE=2 SV=1	ZN827_HUMAN	119089,9844	6,43
Zinc finger protein Eos OS=Homo sapiens GN=IKZF4 PE=1 SV=2	IKZF4_HUMAN	64065,2813	6,38
Zinc finger protein ZIC 5 OS=Homo sapiens GN=ZIC5 PE=1 SV=1	ZIC5_HUMAN	65807,8906	8,86

Supplemental Table 2: List of most frequent identified peptides.

Protein Name	Peptide Sequence	Individuals (n=10)
Salivary acidic proline-rich phosphoprotein ½	GPPPPPPGKPQGPPPQGGRPQ	10
Salivary acidic proline-rich phosphoprotein ½	GPPQQGGHPPPPQGRPQ	10
Salivary acidic proline-rich phosphoprotein ½	GPPQQGGHPRPP	10
Submaxillary gland androgen-regulated protein 3B	FVPPPPPPPYGPGRIPPPPPAPY	10
Submaxillary gland androgen-regulated protein 3B	GPGRIPPPPPAPY	10
Submaxillary gland androgen-regulated protein 3B	GPGRIPPPPPAPYGPGIFPPPPPQP	10
Submaxillary gland androgen-regulated protein 3B	GPYPPGPLAPPQPF	10
Submaxillary gland androgen-regulated protein 3B	GPYPPGPLAPPQPFGPG	10
Submaxillary gland androgen-regulated protein 3B	RGPYPPGPLAPPQPF	10
Submaxillary gland androgen-regulated protein 3B	RIPPPPPAPY	8
Basic salivary proline-rich protein 1	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	8
Basic salivary proline-rich protein 3	GPPPPPQGGRPH	8
Salivary acidic proline-rich phosphoprotein ½	GRPQGPPQQGGHQQ	8

Supplemental Table 3: Contingency table for N-terminal of identified peptides in controls.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total	%
A	3	1	1	1	0	6	1	2	1	0	0	0	2	4	5	1	3	2	0	0	**33**	**2,29**
C	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	**2**	**0,15**
D	0	0	1	0	0	5	0	1	0	0	1	0	2	1	1	0	0	1	0	0	**13**	**0,91**
E	4	0	1	4	0	56	0	0	2	2	0	1	4	1	7	4	2	2	1	0	**90**	**6,32**
F	0	0	0	2	0	19	2	2	0	1	0	2	3	1	0	0	2	8	0	6	**46**	**3,22**
G	6	0	2	6	19	1	0	5	11	14	2	2	43	6	9	2	2	5	0	2	**134**	**9,40**
H	0	0	0	0	0	1	0	0	0	1	2	0	2	0	9	0	0	0	0	0	**16**	**1,11**
I	1	0	0	0	2	4	0	0	1	0	1	0	1	0	1	1	1	0	0	0	**12**	**0,84**
K	2	0	1	0	0	11	1	0	3	0	0	0	26	4	1	2	1	2	0	0	**55**	**3,83**
L	7	0	0	0	1	4	0	1	1	1	0	2	9	1	6	1	0	0	0	3	**39**	**2,70**
M	0	0	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**3**	**0,24**
N	0	0	1	0	0	0	0	0	1	0	1	1	2	2	0	0	0	0	0	0	**9**	**0,60**
P	8	1	0	7	3	40	0	2	5	5	2	0	58	39	52	8	2	7	0	7	**249**	**17,43**
Q	8	0	0	3	0	470	0	0	1	0	0	0	3	8	2	16	1	1	0	5	**520**	**36,36**
R	3	0	2	2	0	13	0	11	2	0	1	0	3	2	2	24	0	0	0	0	**66**	**4,62**
S	7	0	1	1	1	7	0	2	1	0	1	1	3	3	21	0	0	0	5	0	**53**	**3,68**
T	1	0	0	0	1	2	0	0	0	0	0	0	4	0	1	1	1	0	1	0	**11**	**0,78**
V	0	0	2	0	0	2	0	1	1	1	0	0	3	0	0	2	2	0	2	0	**15**	**1,04**
W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0,03**
Y	0	0	1	0	0	52	0	0	0	0	0	0	0	3	6	0	0	0	0	0	**63**	**4,44**
Total	**51**	**3**	**13**	**25**	**26**	**696**	**5**	**26**	**31**	**24**	**11**	**10**	**168**	**76**	**124**	**63**	**17**	**28**	**8**	**24**	**1429**	
%	**3,55**	**0,18**	**0,90**	**1,78**	**1,83**	**48,68**	**0,36**	**1,83**	**2,17**	**1,71**	**0,78**	**0,71**	**11,77**	**5,29**	**8,65**	**4,41**	**1,22**	**1,97**	**0,55**	**1,67**		

The amino acids are represented in one letter code

Supplemental Table 4: Contingency table for C-terminal of identified peptides in controls.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total	%
A	3	0	5	0	0	13	0	0	4	0	0	0	24	2	2	1	0	0	0	0	**55**	**3,64**
C	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	**2**	**0,12**
D	0	0	0	3	0	12	0	0	2	1	0	0	5	1	1	0	0	1	0	0	**28**	**1,84**
E	0	0	0	1	0	1	0	1	2	1	0	0	10	2	3	2	0	2	0	0	**25**	**1,68**
F	0	0	0	0	0	13	4	3	1	0	0	0	5	0	1	0	0	9	0	0	**37**	**2,43**
G	1	0	0	0	7	10	1	0	6	0	0	2	95	2	13	0	2	1	0	3	**143**	**9,51**
H	0	0	0	2	0	0	1	0	0	0	0	0	6	1	2	1	0	2	0	0	**14**	**0,90**
I	0	0	0	0	0	3	0	0	0	0	0	0	6	0	0	0	0	0	0	0	**9**	**0,61**
K	2	0	0	0	5	1	3	2	1	1	0	0	27	0	0	4	0	2	0	0	**50**	**3,30**
L	8	0	0	0	3	0	0	2	2	0	0	1	22	0	1	1	0	1	0	2	**43**	**2,83**
M	0	0	0	1	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	**6**	**0,39**
N	0	0	0	0	0	1	0	0	0	0	0	0	8	0	0	1	0	0	0	0	**10**	**0,66**
P	0	0	0	1	0	29	0	1	0	0	0	0	140	22	16	7	0	4	0	8	**229**	**15,23**
Q	10	0	2	0	3	489	0	0	0	0	0	0	13	14	2	61	0	0	0	1	**595**	**39,59**
R	4	0	1	0	0	35	1	8	4	2	0	1	18	1	0	49	0	0	0	0	**125**	**8,31**
S	1	0	2	0	1	1	1	0	0	0	0	1	24	14	0	0	1	1	0	0	**47**	**3,10**
T	0	0	0	0	0	4	0	1	0	2	0	0	7	0	1	1	0	1	0	0	**16**	**1,05**
V	0	0	0	0	0	3	0	0	0	0	0	0	23	0	0	0	0	1	0	0	**27**	**1,78**
W	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	**2**	**0,11**
Y	0	0	0	0	0	34	0	0	0	0	0	1	8	2	0	0	0	0	0	0	**44**	**2,91**
Total	**30**	**0**	**10**	**8**	**20**	**652**	**11**	**19**	**23**	**7**	**0**	**5**	**446**	**61**	**43**	**128**	**2**	**25**	**0**	**14**	**1504**	
%	**1,97**	**0,00**	**0,68**	**0,52**	**1,30**	**43,38**	**0,72**	**1,25**	**1,56**	**0,49**	**0,00**	**0,33**	**29,63**	**4,06**	**2,83**	**8,53**	**0,16**	**1,64**	**0,00**	**0,96**		

The amino acids are represented in one letter code

Supplemental Table 5: Contingency table for N-terminal of identified peptides in diabetic patients.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total	%
A	5	1	5	2	0	22	5	4	3	3	0	0	9	10	5	3	1	4	0	0	**83**	**3,01**
C	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	**2**	**0,08**
D	0	0	1	0	0	9	0	1	2	1	1	0	1	0	5	1	0	2	0	1	**27**	**0,97**
E	2	0	2	1	1	118	0	1	9	0	0	0	9	4	10	8	1	3	0	0	**167**	**6,10**
F	0	0	1	2	0	35	1	16	0	0	0	0	4	0	1	0	0	7	0	70	**138**	**5,02**
G	28	1	11	17	40	18	0	8	16	20	1	6	77	13	17	7	3	9	0	16	**308**	**11,22**
H	0	1	0	0	0	0	7	2	1	1	0	0	2	1	25	19	0	0	0	0	**62**	**2,25**
I	1	0	0	2	1	3	0	0	2	1	2	0	1	1	2	1	1	0	0	2	**20**	**0,74**
K	6	0	0	1	4	23	1	0	0	0	2	0	4	0	17	2	12	6	0	0	**80**	**2,92**
L	15	1	1	1	0	6	0	0	7	1	0	3	10	0	3	0	2	2	0	29	**83**	**3,04**
M	1	0	0	0	0	5	0	0	0	1	1	0	1	1	0	0	0	0	0	0	**10**	**0,37**
N	1	0	0	0	0	0	0	0	2	1	0	0	1	2	0	1	0	0	0	3	**13**	**0,46**
P	17	0	0	8	6	97	4	2	6	6	1	0	123	50	64	22	9	7	0	14	**436**	**15,91**
Q	13	0	0	4	0	678	0	1	1	1	0	0	17	17	8	32	1	2	0	40	**816**	**29,76**
R	2	0	3	5	1	29	1	6	7	1	1	1	11	1	6	38	2	1	1	0	**118**	**4,29**
S	21	0	2	3	1	1	3	1	4	6	1	13	3	4	15	5	0	0	4	1	**88**	**3,22**
T	2	0	2	2	5	3	0	0	5	2	0	0	4	0	2	2	0	6	0	0	**34**	**1,25**
V	1	0	1	3	1	2	1	1	0	1	1	0	7	1	2	2	1	0	1	1	**29**	**1,04**
W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0,01**
Y	1	0	10	0	0	144	0	1	7	4	0	0	2	26	31	0	2	0	0	0	**229**	**8,33**
Total	**116**	**5**	**41**	**52**	**60**	**1193**	**23**	**45**	**71**	**53**	**11**	**23**	**288**	**133**	**213**	**144**	**37**	**51**	**6**	**176**	**2743**	
%	**4,21**	**0,20**	**1,49**	**1,90**	**2,19**	**43,50**	**0,84**	**1,66**	**2,57**	**1,93**	**0,42**	**0,83**	**10,51**	**4,85**	**7,75**	**5,26**	**1,36**	**1,86**	**0,23**	**6,43**		

The amino acids are represented in one letter code

Supplemental Table 6: Contingency table for C-terminal of identified peptides in diabetic patients.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total	%
A	7	0	15	0	0	22	2	0	10	0	0	0	45	0	0	2	0	1	0	0	**105**	**3,54**
C	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	**2**	**0,08**
D	1	0	0	4	0	10	0	13	0	0	0	0	3	0	3	7	1	3	0	0	**45**	**1,51**
E	0	0	0	6	0	1	1	0	16	1	0	4	41	4	4	0	0	1	2	0	**81**	**2,73**
F	0	0	0	2	0	36	32	7	0	6	0	0	21	0	0	0	0	4	0	7	**116**	**3,93**
G	2	0	1	0	11	15	2	1	8	3	0	1	165	2	26	0	1	2	0	10	**250**	**8,45**
H	0	0	0	18	5	0	24	0	0	0	0	0	10	0	10	22	0	4	0	1	**94**	**3,17**
I	0	0	4	0	0	6	0	0	0	1	0	1	10	1	0	0	0	0	0	0	**25**	**0,85**
K	2	0	0	0	39	2	40	0	1	0	0	3	46	0	6	12	0	4	0	0	**156**	**5,26**
L	12	0	0	0	0	1	3	1	1	2	0	2	59	0	1	2	0	1	0	20	**106**	**3,59**
M	0	0	1	0	0	1	0	0	2	0	0	0	0	0	0	0	1	0	0	0	**6**	**0,19**
N	0	0	0	0	0	0	0	0	1	0	0	1	7	2	0	0	0	2	0	0	**13**	**0,43**
P	5	1	0	1	0	57	3	1	3	3	0	1	281	33	28	11	0	10	0	15	**452**	**15,28**
Q	11	0	4	1	7	728	0	0	1	1	0	0	36	14	4	103	0	1	0	7	**917**	**30,97**
R	3	0	1	2	0	66	5	39	34	1	0	0	32	0	14	89	0	0	0	0	**287**	**9,68**
S	6	0	8	0	0	12	16	0	0	0	0	1	41	30	1	4	1	2	0	0	**122**	**4,12**
T	0	0	0	0	0	7	0	0	2	2	0	0	19	1	0	0	0	3	0	0	**35**	**1,18**
V	1	0	0	0	0	2	1	1	0	2	0	1	37	1	0	0	0	0	0	0	**48**	**1,63**
W	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	**1**	**0,04**
Y	0	0	0	0	0	82	0	0	1	0	0	0	6	11	0	0	0	0	0	0	**100**	**3,38**
Total	**50**	**1**	**35**	**34**	**63**	**1048**	**129**	**65**	**82**	**23**	**0**	**16**	**860**	**100**	**97**	**252**	**4**	**40**	**2**	**60**	**2961**	
%	**1,68**	**0,02**	**1,20**	**1,14**	**2,13**	**35,40**	**4,36**	**2,19**	**2,78**	**0,77**	**0,01**	**0,53**	**29,05**	**3,36**	**3,27**	**8,52**	**0,14**	**1,36**	**0,07**	**2,03**		

The amino acids are represented in one letter code

Supplemental Table 7: Cleavage site frequency for N-terminal of identified SGP.

%	T1D 1	T1D 2	T1D 3	T1D 4	T1D 5	Ctrl 1	Ctrl 2	Ctrl 3	Ctrl 4	Ctrl 5
AG	0,37	0,00	0,00	0,00	0,00	0,00	0,65	0,51	0,00	0,00
AH	0,00	0,00	0,39	0,27	0,44	0,33	0,00	0,00	0,15	0,00
AP	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
AQ	0,37	0,00	0,29	0,40	0,22	0,00	0,00	0,51	0,39	0,33
AR	0,00	0,00	0,29	0,00	0,44	0,00	0,00	0,00	0,39	0,67
AS	0,00	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00
DL	0,00	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00
DG	0,00	0,00	0,00	0,00	0,00	0,83	0,00	0,00	0,00	0,00
DM	0,00	0,00	0,00	0,00	0,00	0,33	0,00	0,00	0,00	0,00
EG	15,75	0,00	5,42	7,47	4,09	19,20	9,09	4,57	0,00	3,01
EK	0,00	0,00	0,00	0,00	0,26	0,00	0,00	0,00	0,00	0,00
EQ	0,00	0,00	0,29	0,00	0,22	0,00	0,00	0,00	0,00	0,00
ER	0,73	0,00	0,59	0,81	0,44	1,67	0,00	1,52	0,00	0,67
ES	0,37	0,00	0,29	0,40	0,22	0,83	0,00	0,51	0,00	0,33
FG	2,50	2,61	1,32	1,88	1,25	2,50	2,60	2,54	1,16	1,34
FH	0,00	0,00	0,00	0,00	0,18	0,00	0,00	0,00	0,31	0,27
FI	0,00	0,00	0,93	0,54	1,29	0,67	0,00	0,00	0,39	0,00
FP	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,51	0,00	0,00
FV	1,71	0,00	0,00	0,00	0,00	0,83	0,65	2,54	0,00	0,00
FY	1,28	8,47	3,76	3,30	2,58	0,00	0,00	0,00	1,23	0,94
GF	1,71	0,45	1,02	2,36	1,29	0,83	0,65	2,03	1,54	1,34
GG	0,00	0,45	0,00	0,47	2,39	0,00	0,00	0,00	0,00	0,00
GI	0,43	0,00	0,00	0,47	0,26	0,83	0,00	0,00	0,39	0,33
GK	0,00	1,53	0,29	1,21	0,00	0,00	0,65	0,00	0,00	3,34
GL	0,24	0,00	0,59	0,00	0,59	0,00	0,00	0,00	0,77	0,54
GN	0,00	0,38	0,00	0,00	0,22	0,00	0,00	0,00	0,00	0,33
GP	1,28	0,38	2,93	3,23	0,26	0,00	0,65	4,06	3,08	0,00
GQ	0,00	0,00	0,00	0,00	0,26	0,00	1,30	0,00	0,00	0,00
GR	0,43	0,00	0,00	0,94	0,26	0,00	1,95	1,52	0,00	0,00
GY	0,67	1,15	0,49	0,40	1,03	0,00	0,00	0,00	0,15	0,27
HA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00
HQ	0,00	0,00	0,00	0,00	0,00	0,33	0,00	0,00	0,00	0,00

HH	0,24	0,00	0,00	0,81	0,63	0,00	0,00	0,00	0,00	0,00
HP	0,00	0,00	0,00	0,00	0,26	0,00	0,00	0,00	0,00	0,00
HR	2,56	0,38	0,93	1,75	1,25	2,50	0,65	1,02	0,00	0,67
HS	1,47	0,00	0,59	0,81	1,51	0,00	0,00	0,00	0,00	0,00
KA	0,00	0,00	0,10	0,00	0,15	0,00	0,00	0,00	0,46	0,13
KC	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00
KE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00
KF	1,04	0,00	0,24	0,00	0,00	0,00	0,00	0,00	0,15	0,00
KH	0,00	0,00	0,24	0,00	0,00	0,00	0,00	0,00	0,31	0,13
KL	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
KP	0,00	0,00	0,29	2,83	1,40	0,83	4,55	0,00	1,93	2,01
KR	0,31	0,00	0,73	0,67	0,74	0,00	0,00	0,00	0,31	0,13
KS	0,24	0,00	0,20	0,00	0,00	0,00	0,65	0,00	0,15	0,00
LA	1,10	0,00	0,73	1,01	0,70	1,17	0,00	0,00	0,54	0,47
LG	0,00	0,00	0,10	0,00	0,52	0,00	0,00	0,00	0,31	0,00
LP	0,43	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
LY	0,37	3,37	1,46	1,21	1,29	0,00	0,00	0,00	0,93	0,27
MP	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
NK	0,00	0,45	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00
NN	0,00	0,00	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00
NP	0,00	0,00	0,00	0,00	0,00	0,83	0,00	0,00	0,00	0,00
NQ	0,00	0,76	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,67
NY	0,00	0,89	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PA	0,43	0,19	0,15	1,01	0,11	0,83	0,65	1,02	0,00	0,33
PF	0,43	0,00	0,68	0,00	0,26	0,00	0,00	0,00	0,46	0,27
PG	0,00	2,55	0,59	0,47	1,29	0,00	0,00	0,00	0,39	0,47
PH	0,00	0,00	0,59	0,00	0,00	0,33	0,00	0,00	0,00	0,00
PI	0,18	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PL	0,43	0,00	0,00	0,00	0,00	0,00	0,00	0,51	0,31	0,60
PM	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
PP	1,77	13,30	0,29	8,21	1,51	0,83	3,90	4,06	3,62	5,69
PQ	0,79	1,72	1,56	5,45	2,58	1,67	5,84	3,55	1,54	4,68
PR	3,36	3,06	2,78	3,90	2,54	4,17	2,60	6,60	5,94	3,34
PS	0,00	0,00	0,59	0,00	0,33	0,00	0,00	0,00	1,16	1,00

PY	0,85	0,76	0,59	0,47	0,48	0,00	0,65	1,02	0,85	0,74
QA	0,73	0,00	2,34	0,00	0,00	0,00	1,30	1,02	1,54	0,00
QE	0,00	0,00	0,88	0,00	0,15	0,00	0,00	1,52	0,00	0,00
QG	34,98	19,03	42,75	26,85	42,99	37,56	44,16	42,13	49,34	47,83
QI	0,00	0,00	0,00	0,27	0,00	0,00	0,00	0,00	0,00	0,00
QL	0,18	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
QP	0,00	0,00	0,00	0,00	0,52	0,00	1,30	0,00	0,39	0,00
QQ	2,93	0,00	0,00	0,87	0,55	0,83	0,00	3,05	0,39	0,13
QR	0,43	0,00	0,00	0,87	0,74	0,00	0,65	0,00	0,39	0,00
QS	2,50	0,76	0,93	3,03	1,47	3,34	1,30	1,52	1,16	1,34
QY	0,73	4,20	0,59	2,02	2,65	0,00	0,00	0,00	1,39	0,54
RA	0,00	0,00	0,10	0,00	0,07	0,00	0,00	0,00	0,85	0,13
RD	0,00	0,00	0,10	0,00	0,07	0,00	0,00	0,00	0,31	0,13
RE	0,00	0,00	0,68	0,00	0,00	0,00	0,00	0,00	0,15	0,27
RG	0,00	0,00	0,83	0,47	0,26	0,83	0,65	0,51	0,69	0,74
RH	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00
RI	1,28	0,00	0,00	0,67	0,00	0,00	3,25	2,54	0,08	0,33
RK	0,24	0,00	1,17	0,00	0,18	0,00	0,00	0,00	0,31	0,27
RP	0,00	0,76	0,00	0,00	0,00	0,83	0,00	0,00	0,00	0,00
RQ	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,51	0,00	0,00
RR	0,00	0,00	0,68	0,00	0,00	0,00	0,00	0,00	0,39	0,27
RS	0,00	0,00	5,08	0,81	2,21	3,34	1,30	0,51	2,31	3,41
SA	2,99	0,00	0,34	0,94	0,52	0,83	0,65	1,52	0,39	0,00
SE	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
SF	0,00	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00
SG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00
SH	0,00	0,00	0,00	0,00	0,63	0,00	0,00	0,00	0,00	0,00
SK	0,00	0,00	0,59	0,00	0,00	0,00	0,00	0,00	0,00	0,33
SN	0,00	4,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SP	0,00	0,00	0,00	0,00	0,00	0,83	0,00	0,00	0,39	0,00
SQ	0,00	0,76	0,29	0,00	0,00	0,00	0,00	0,51	0,39	0,33
SR	1,47	0,00	1,46	0,81	0,22	1,67	1,30	1,02	1,54	3,68
SS	0,12	0,38	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00
SV	0,12	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

TF	0,00	0,76	0,00	0,40	0,22	0,00	0,00	0,00	0,31	0,00
TL	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TP	0,00	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00
VF	0,00	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00
VS	0,12	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
YD	0,00	2,67	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
YG	5,07	20,31	6,05	3,70	5,04	7,51	5,19	5,08	5,55	3,61
YK	0,00	0,00	0,49	0,34	0,70	0,00	0,00	0,00	0,00	0,00
YL	0,61	0,45	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00
YP	0,00	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00
YQ	0,73	3,06	0,00	2,02	1,55	0,00	0,00	0,00	0,46	0,27
YR	0,92	0,00	1,95	2,15	2,36	0,00	0,00	0,00	1,23	1,07
YT	0,00	0,00	0,00	0,00	0,44	0,00	0,00	0,00	0,15	0,00

Supplemental Table 8: Cleavage site frequency for C-terminal of identified SGP.

%	T1D 1	T1D 2	T1D 3	T1D 4	T1D 5	Ctrl 1	Ctrl 2	Ctrl 3	Ctrl 4	Ctrl 5
AD	0,29	0,00	1,18	1,03	0,89	0,00	0,00	0,00	0,85	0,74
AG	0,35	0,00	0,26	0,36	0,20	0,00	0,00	0,99	1,07	0,93
AK	0,29	0,00	1,09	0,00	0,49	0,00	0,00	0,00	0,85	0,37
AP	0,81	0,32	0,00	0,97	0,46	0,00	0,00	0,99	1,07	0,62
AS	0,00	0,00	0,09	0,00	0,07	0,00	0,00	0,00	0,28	0,12
DE	0,81	0,00	0,00	0,00	0,00	0,00	0,62	0,99	0,00	0,00
DG	0,40	0,00	0,61	0,85	0,00	0,75	0,00	1,49	0,36	0,62
DR	0,17	0,00	0,00	0,00	0,10	0,00	0,00	0,00	0,00	0,00
DS	0,00	0,00	0,96	0,30	0,33	0,00	0,00	0,00	0,00	0,00
EE	0,81	0,00	0,00	0,00	0,00	0,00	0,62	0,00	0,00	0,00
EG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,50	0,00	0,00
EK	0,00	3,03	0,00	0,00	0,63	0,00	0,00	0,00	0,00	0,00
EL	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00
EQ	0,00	0,32	0,00	0,00	0,43	0,00	0,00	0,50	0,00	0,00
ER	0,81	0,00	0,00	0,00	0,00	0,75	0,00	0,99	0,00	0,00
ES	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,36	0,31
FE	0,00	0,00	0,17	0,24	0,13	0,00	0,00	0,00	0,00	0,00
FG	1,56	2,16	1,14	1,93	1,51	1,50	1,85	0,99	1,14	0,74
FH	1,27	3,78	1,57	0,73	0,56	0,00	0,00	0,00	0,71	0,49
FI	0,81	0,00	0,00	0,00	0,69	0,00	0,00	0,50	0,36	0,31
FL	0,35	0,32	0,26	0,00	0,39	0,00	0,00	0,00	0,00	0,12
FP	0,00	0,38	0,31	0,85	0,23	0,00	0,00	0,00	0,00	0,00
FV	0,81	0,00	0,00	0,42	0,00	0,00	1,85	1,49	0,71	0,31
FY	0,40	0,76	0,00	0,42	0,23	0,00	0,00	0,00	0,14	0,00
GF	0,81	0,76	0,31	0,85	0,23	0,75	1,23	0,99	0,36	0,31
GG	0,81	0,00	0,00	0,85	1,51	1,50	0,62	0,00	2,13	0,00
GH	0,00	0,00	0,00	0,00	0,00	0,75	0,00	0,00	0,00	0,00
GK	0,81	0,00	0,00	0,00	0,46	0,00	0,00	0,00	1,07	0,00
GL	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00
GN	0,00	0,00	0,26	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GP	2,02	0,32	0,83	1,33	1,71	4,50	2,47	1,98	0,50	2,78

GR	1,04	1,95	0,79	0,85	0,36	0,75	1,23	1,98	0,99	0,62
GY	0,00	0,00	0,22	0,67	1,05	0,00	0,00	0,00	0,43	0,37
HE	0,29	2,27	0,83	0,73	0,39	0,00	0,00	0,00	0,43	0,12
HF	0,00	0,00	0,52	0,00	0,26	0,00	0,00	0,00	0,00	0,00
HH	0,40	3,03	1,14	0,42	0,86	0,00	0,00	0,00	0,14	0,12
HP	0,40	0,00	0,00	0,36	0,10	0,00	0,00	0,00	0,00	0,00
HR	0,40	1,89	0,00	0,00	0,23	0,00	0,62	0,50	0,00	0,00
HS	0,40	3,41	0,61	0,42	0,56	0,00	0,00	0,00	0,14	0,12
HY	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00
ID	1,21	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IG	0,00	0,65	0,26	0,00	0,20	0,00	0,00	0,00	0,14	0,25
IP	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IQ	0,00	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,14	0,00
KA	0,00	0,00	0,17	0,00	0,13	0,00	0,00	0,00	0,57	0,25
KF	1,39	3,03	2,93	0,73	1,18	0,00	0,00	0,00	0,85	0,74
KH	0,69	6,43	1,27	1,03	0,56	0,00	0,00	0,00	0,57	0,49
KN	0,00	0,00	0,00	0,24	0,00	0,30	0,00	0,00	0,00	0,00
KP	0,40	1,68	1,31	4,35	0,59	1,50	6,79	0,50	0,00	1,23
KR	0,00	0,00	0,00	0,00	0,82	0,00	0,00	0,00	0,00	0,12
KS	0,46	0,00	1,05	0,73	0,53	2,70	0,00	0,00	0,28	0,00
LA	1,27	0,00	0,61	0,42	0,59	0,75	0,62	0,99	0,50	0,93
LF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,21	0,00
LI	0,00	0,00	0,26	0,00	0,00	0,00	0,00	0,00	0,36	0,31
LN	0,23	0,00	0,00	0,00	0,13	0,30	0,00	0,00	0,00	0,00
LP	0,40	0,00	0,00	0,00	0,10	0,00	0,00	0,00	0,00	0,00
LS	0,23	0,00	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00
LV	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,12
LY	0,35	0,97	0,26	1,09	1,78	0,00	0,00	0,00	0,71	0,12
ML	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14	0,00
MT	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00
NP	0,00	0,00	0,26	0,18	0,00	0,30	1,23	0,00	0,43	0,31
NQ	0,00	0,00	0,00	0,00	0,23	0,00	0,00	0,00	0,00	0,00
PE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14	0,00
PG	1,21	0,38	0,31	1,27	1,84	1,50	1,85	2,48	0,71	0,31

PH	0,17	0,00	0,00	0,00	0,23	0,00	0,00	0,00	0,00	0,00
PP	3,29	10,86	6,51	9,43	1,65	1,50	4,94	10,40	7,03	4,63
PQ	1,10	1,35	2,10	2,54	0,30	1,50	4,32	2,97	0,36	1,85
PR	1,21	0,76	0,92	1,27	1,38	1,50	2,47	1,98	0,71	0,62
PS	0,00	0,32	0,52	0,36	0,99	0,00	0,00	0,50	1,07	0,93
PV	0,69	0,00	0,00	0,36	0,20	0,00	0,00	0,00	0,28	0,25
PY	0,81	0,38	0,61	0,85	0,86	0,00	0,00	0,99	1,14	0,74
QA	0,69	0,00	0,52	0,73	0,39	1,50	1,23	0,99	0,71	0,62
QD	0,00	0,38	0,00	0,42	0,23	0,00	0,00	0,00	0,36	0,31
QE	0,00	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00
QF	0,81	0,00	0,00	0,42	0,46	0,00	0,62	0,50	0,36	0,00
QG	33,53	19,78	43,92	27,75	38,60	36,04	45,06	42,57	44,74	47,04
QL	0,00	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00
QP	0,35	0,32	0,52	0,36	0,99	0,00	0,00	0,00	0,00	2,78
QQ	1,62	0,38	0,31	0,85	0,39	2,25	1,85	2,97	0,36	0,00
QR	0,40	0,00	0,00	0,36	0,20	0,00	0,62	0,00	0,00	0,00
QS	13,53	0,32	3,98	5,44	2,93	15,77	4,32	6,44	1,42	4,94
QY	0,00	0,65	0,26	0,36	0,39	0,00	0,00	0,00	0,14	0,12
RA	0,35	0,00	0,17	0,00	0,13	0,00	0,00	0,00	0,43	0,74
RD	0,00	0,00	0,09	0,00	0,07	0,00	0,00	0,00	0,14	0,12
RE	0,23	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00
RF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,12
RG	3,24	1,51	2,67	3,26	3,62	5,26	1,85	3,47	2,98	2,28
RH	0,81	0,00	0,22	0,00	0,23	0,00	0,00	0,00	0,28	0,12
RI	1,10	5,57	1,05	1,09	1,02	0,75	0,62	0,50	1,07	0,74
RK	0,69	1,14	2,84	2,48	0,95	0,00	0,00	0,00	0,57	0,62
RL	0,00	0,00	0,09	0,00	0,13	0,00	0,00	0,00	0,28	0,25
RP	3,12	0,00	0,00	0,79	0,92	3,00	1,85	0,50	1,07	0,93
RQ	0,12	0,00	0,00	0,00	0,00	0,75	0,00	0,00	0,00	0,00
RR	0,00	1,78	0,31	0,00	0,89	0,00	0,00	0,00	0,00	0,00
RS	0,00	2,27	5,24	2,90	7,70	0,00	0,62	3,47	7,10	6,48
SA	0,40	0,00	0,00	0,18	0,23	0,00	0,00	0,00	0,00	0,00
SD	0,40	0,00	0,00	1,27	0,46	1,50	0,00	0,00	0,00	0,00
SG	0,75	0,00	0,00	1,15	0,63	0,00	0,00	0,00	0,00	0,00

SH	0,40	2,65	0,31	0,42	0,39	0,00	0,00	0,00	0,14	0,12
SP	0,00	2,59	0,52	1,45	0,20	0,00	0,00	0,00	0,50	5,25
SQ	1,21	0,65	0,00	3,39	1,91	5,26	4,32	0,00	0,00	0,00
SS	0,00	0,00	0,17	0,00	0,46	0,00	0,00	0,00	0,00	0,00
ST	0,00	0,00	0,00	0,18	0,00	0,00	0,00	0,00	0,00	0,00
SV	0,00	0,00	0,09	0,00	0,00	0,00	0,00	0,00	0,14	0,00
TP	0,17	0,00	0,00	0,18	0,00	0,00	0,00	0,00	0,00	0,00
VP	0,00	0,76	0,61	0,00	1,02	0,00	0,00	0,00	0,71	0,00
YG	2,77	6,76	3,32	3,99	3,03	3,75	3,09	2,48	4,69	1,67
YK	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00
YP	0,40	0,00	0,00	0,42	0,23	1,05	0,62	0,50	0,00	0,31
YQ	0,00	0,97	0,26	0,73	0,59	0,00	0,00	0,00	0,43	0,12

Supplemental Table 9: Cleavage site frequency for N-terminal of identified NSGP.

%	T1D 1	T1D 2	T1D 3	T1D 4	T1D 5	Ctrl 1	Ctrl 2	Ctrl 3	Ctrl 4	Ctrl 5
AA	0,33	1,54	0,00	0,25	0,87	0,00	0,79	1,54	0,25	1,13
AC	0,00	0,00	0,83	0,00	0,00	0,00	0,79	0,00	0,98	0,00
AD	1,15	0,90	0,83	0,49	0,11	0,00	0,00	1,54	0,00	0,00
AE	0,00	0,00	0,96	0,16	0,22	0,00	0,00	0,00	0,74	0,00
AG	2,14	3,22	0,83	2,22	3,57	2,70	1,32	0,00	0,98	1,13
AI	0,99	0,00	0,00	0,99	0,00	0,00	0,00	0,00	0,00	1,28
AK	0,00	1,80	0,00	0,00	0,00	0,00	0,00	0,00	0,74	0,00
AL	0,49	0,00	0,83	0,41	0,22	0,00	0,00	0,00	0,00	0,00
AM	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
AN	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00
AP	0,66	0,99	1,32	1,15	2,96	0,00	0,00	0,92	1,72	0,28
AQ	0,00	2,32	0,00	0,00	0,65	0,00	0,00	0,00	1,23	0,00
AR	0,00	0,00	0,41	0,16	0,00	2,70	0,79	0,00	0,00	0,00
AS	0,16	0,00	0,69	0,00	0,76	0,00	0,79	0,00	0,00	0,00
AT	0,00	0,90	0,00	0,00	0,00	0,00	0,00	1,54	0,74	1,28
AV	0,99	0,00	0,00	1,07	0,11	0,00	0,00	0,00	0,98	0,57
AY	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
CA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00
CD	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,00	0,00
CH	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
CI	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
CL	0,00	0,00	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
CN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
CS	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
CT	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
DD	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,57
DG	0,33	1,81	1,15	1,97	2,30	0,00	0,00	0,00	1,97	1,84
DI	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,57
DK	0,00	0,51	0,00	0,49	0,22	0,00	0,00	0,00	0,00	0,28
DL	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
DM	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,43
DP	0,00	0,00	0,83	0,08	0,00	2,16	0,00	0,00	1,47	0,00

DQ	0,00	0,00	0,00	0,00	0,00	0,00	1,06	0,00	0,74	0,00
DR	0,82	0,00	0,55	0,49	0,97	0,00	0,00	0,00	0,00	0,43
DS	0,00	0,00	0,00	0,16	0,32	0,00	0,00	0,00	0,00	0,00
DV	0,00	0,00	0,00	0,99	0,00	0,00	1,06	0,00	0,00	0,00
DY	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EA	0,16	0,00	0,00	0,08	0,65	0,00	1,06	1,85	0,00	1,28
ED	0,00	0,00	0,83	0,49	0,00	0,00	0,00	0,00	0,98	0,00
EE	0,00	0,00	0,00	0,16	0,11	0,00	2,11	2,46	0,00	0,28
EF	0,00	0,51	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00
EG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,92	0,00	0,57
EI	0,99	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EK	0,99	0,00	1,65	0,49	1,30	0,00	0,00	0,00	0,00	1,70
EL	0,00	0,00	0,00	0,00	0,00	0,00	1,06	1,23	0,00	0,00
EN	0,00	0,00	0,00	0,00	0,00	0,00	1,06	0,00	0,00	0,00
EP	0,00	1,80	1,38	1,56	0,00	3,78	0,00	2,77	0,00	0,28
EQ	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,57
ER	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
ES	0,00	0,00	0,83	0,99	0,00	0,00	0,00	0,00	0,74	0,57
ET	0,00	0,00	0,69	0,00	0,00	0,00	2,11	0,00	0,00	0,43
EV	0,33	0,00	0,00	0,49	0,65	0,00	0,53	1,23	0,00	0,43
EW	0,00	0,00	0,00	0,00	0,00	0,00	1,06	0,00	0,00	0,00
FD	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
FE	0,00	0,00	0,00	0,49	0,65	0,00	0,00	0,00	0,98	0,57
FG	0,00	0,00	0,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00
FI	0,82	0,00	0,69	0,49	0,00	0,00	0,00	0,00	0,00	0,00
FK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,28
FL	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,14
FN	0,16	0,00	0,00	0,00	0,00	0,00	1,06	1,23	0,00	0,00
FP	0,49	0,77	0,00	0,58	0,43	1,08	0,00	2,46	0,00	0,00
FQ	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
FR	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
FS	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
FT	0,00	0,00	0,00	0,00	0,00	2,70	0,00	1,23	0,00	0,00
FV	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,57

GA	3,13	7,34	2,48	3,45	0,32	0,00	0,00	0,00	1,72	2,98
GC	0,00	0,00	0,28	0,08	0,00	0,00	0,00	0,00	0,00	0,00
GD	0,99	2,06	0,00	1,97	0,87	0,00	1,06	0,00	0,00	0,57
GE	0,00	3,86	2,61	2,55	0,32	0,00	0,79	2,46	0,74	2,41
GF	1,32	0,51	0,83	1,31	4,55	0,00	1,32	0,00	3,69	0,43
GG	0,00	0,00	0,83	0,49	0,11	0,00	0,00	0,00	0,98	0,00
GI	0,00	1,67	0,00	0,33	0,32	0,00	0,00	0,00	0,00	1,13
GK	1,15	0,00	0,00	0,66	1,95	0,00	0,00	0,00	0,49	0,00
GL	0,00	1,80	3,03	1,64	1,19	0,00	2,64	3,08	0,25	4,11
GM	0,49	0,00	0,00	0,25	0,00	0,00	0,00	0,00	0,00	1,28
GN	0,00	0,77	1,38	0,16	0,00	0,00	1,06	0,00	0,00	0,14
GP	5,26	7,21	7,98	3,29	6,39	22,16	9,50	4,00	5,16	2,70
GQ	1,97	2,96	0,55	0,49	1,41	0,00	0,00	0,00	0,49	2,41
GR	1,97	1,80	0,00	1,56	1,08	0,00	0,79	2,15	0,98	0,14
GS	0,00	0,00	1,38	1,07	1,19	1,62	0,00	0,00	0,25	0,99
GT	0,00	0,77	0,00	0,58	0,11	0,00	1,32	0,92	0,00	0,00
GV	1,15	2,70	2,20	0,16	0,00	2,70	0,00	1,54	1,23	1,13
GW	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
GY	0,99	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	0,00
HC	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
HG	0,00	0,00	0,00	0,00	0,22	0,00	0,53	0,00	0,00	0,43
HH	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,14
HI	0,00	0,00	0,83	0,49	0,00	0,00	0,00	0,00	0,00	0,00
HK	0,00	0,00	0,00	0,58	0,00	0,00	0,00	0,00	0,00	0,00
HL	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,57
HM	0,00	0,00	0,00	0,00	0,00	0,00	1,32	1,54	0,00	0,00
HP	0,00	0,00	0,00	0,33	0,11	0,00	0,53	0,00	0,00	0,99
HQ	0,00	0,00	0,00	0,58	0,00	0,00	0,00	0,00	0,00	0,00
HR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,71
HS	0,16	0,00	0,00	0,25	0,22	0,00	0,26	0,00	0,00	0,00
HT	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
IA	0,16	0,00	0,28	0,08	0,00	0,00	0,00	0,00	1,23	0,00
IE	0,00	0,00	1,51	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IF	0,00	0,00	0,00	0,25	0,00	0,00	0,00	1,23	0,98	0,00

IG	1,48	0,00	0,00	0,00	0,76	1,62	1,06	0,00	0,49	1,28
IK	0,00	0,00	0,28	0,00	0,65	0,00	0,00	0,92	0,00	0,00
IL	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
IM	0,00	0,00	0,69	0,41	0,00	0,00	0,00	0,00	0,49	0,28
IP	0,49	0,00	0,00	0,33	0,00	0,00	0,00	1,23	0,00	0,43
IQ	0,00	0,90	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IR	0,00	0,00	0,00	0,00	0,87	0,00	1,06	0,00	0,00	0,00
IS	0,33	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,71
IT	0,16	0,00	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,85
IY	0,00	0,00	0,83	0,00	0,43	0,00	0,00	0,00	0,00	0,00
KA	0,66	0,90	0,69	0,25	0,54	0,00	0,00	0,00	0,00	0,00
KC	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
KD	0,00	0,00	0,00	0,00	0,22	0,00	0,00	0,00	1,23	0,00
KE	0,33	0,39	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
KG	2,14	5,28	2,61	2,05	1,62	0,00	3,17	2,15	3,19	3,12
KK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	1,56
KL	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
KM	0,00	0,00	0,69	0,00	0,00	0,00	0,00	0,00	0,00	0,00
KP	1,48	0,00	0,83	0,58	0,00	4,86	1,58	1,85	2,21	0,43
KQ	0,33	0,00	0,00	0,00	0,00	1,62	1,85	0,00	0,00	1,56
KS	1,15	0,00	0,00	0,00	0,22	1,62	0,00	0,00	0,00	0,00
KT	0,00	0,77	0,83	0,00	0,00	0,00	0,79	0,00	0,00	0,00
KV	1,97	0,00	0,00	0,49	0,65	0,00	1,06	2,46	0,00	0,00
LA	0,49	0,00	0,00	0,16	0,22	0,00	0,00	0,00	3,44	0,14
LC	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
LD	0,00	0,64	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
LE	0,33	0,00	0,28	0,08	0,11	0,00	0,00	0,00	0,00	0,00
LF	0,00	0,00	0,00	0,08	0,00	0,00	0,00	1,23	0,00	0,00
LG	0,00	0,64	0,00	0,66	0,11	1,62	0,79	0,00	1,97	0,00
LH	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,14
LI	0,16	0,00	0,00	0,00	0,00	0,00	0,79	0,92	0,00	0,00
LK	1,97	1,03	0,96	0,82	0,00	0,00	0,00	0,00	0,00	0,57
LL	0,16	0,00	0,69	0,08	0,00	0,00	0,00	0,92	0,00	0,43
LN	0,33	0,00	0,96	0,16	0,22	1,08	1,58	0,00	0,00	0,57

LP	0,49	0,00	0,00	1,64	2,06	6,49	1,85	2,77	0,00	2,41
LQ	0,16	0,00	0,00	0,00	0,00	2,70	0,00	0,00	0,00	0,28
LR	0,82	0,00	0,00	0,82	0,22	2,16	3,17	0,00	1,23	1,28
LS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,85	0,00	0,14
LT	0,82	0,00	0,00	0,25	0,32	0,00	0,00	0,00	0,00	0,00
LV	0,33	0,00	0,00	0,16	0,87	0,00	0,26	0,00	0,00	0,00
LY	0,16	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
MA	0,00	0,00	0,69	0,08	0,00	0,00	0,00	0,00	0,00	0,14
MD	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,92	0,00	0,00
MG	0,66	1,16	0,00	0,16	1,19	0,00	0,00	0,00	0,98	0,71
ML	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
MM	0,00	0,00	0,00	0,00	0,43	0,00	0,79	0,00	0,00	0,00
MP	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
MQ	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NA	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NC	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
ND	0,00	0,00	0,00	0,16	0,00	0,00	0,00	1,54	0,00	0,00
NF	0,00	0,00	0,00	0,00	0,00	0,00	0,26	0,00	0,00	0,00
NG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,28
NK	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NL	0,00	0,00	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
NP	0,16	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,43
NS	0,00	0,00	0,69	0,00	0,22	0,00	0,00	0,62	0,00	0,00
NT	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
PA	1,48	0,00	0,55	2,30	1,41	0,00	1,06	1,23	0,49	0,99
PC	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,92	0,00	0,00
PD	0,00	0,00	0,00	0,00	0,00	0,00	0,53	0,00	0,00	0,00
PE	0,16	1,54	0,00	0,82	1,84	0,00	0,79	0,00	1,97	3,40
PF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,74	0,00
PG	13,82	9,52	10,73	6,98	7,58	18,92	11,87	9,23	7,13	6,67
PH	1,15	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
PI	0,00	0,00	0,00	0,00	0,76	0,00	0,00	0,00	2,21	0,43
PK	0,82	2,06	0,00	0,00	0,97	0,00	0,00	1,85	2,21	1,56

PL	0,49	0,00	0,55	1,48	0,00	0,00	0,53	1,23	0,00	0,43
PM	0,00	0,00	0,00	0,00	0,22	2,16	0,00	1,23	0,00	0,57
PP	4,77	1,42	6,74	3,86	8,01	5,41	3,96	2,46	4,42	4,68
PQ	1,48	0,00	0,00	1,15	0,32	0,00	1,58	0,00	0,49	0,71
PR	0,49	0,90	0,00	0,49	1,95	3,24	1,32	1,23	0,74	0,99
PS	0,66	0,90	1,93	2,79	3,25	0,00	0,00	0,00	0,25	1,56
PT	1,97	1,16	0,83	0,90	0,65	0,00	0,00	0,00	2,21	0,43
PV	0,99	0,90	1,93	0,49	0,43	0,00	0,00	1,23	7,13	0,43
PY	0,00	0,00	0,96	0,25	0,00	0,00	0,00	0,00	0,00	0,00
QA	0,00	0,00	0,00	0,08	0,54	0,00	0,00	0,00	0,00	0,14
QC	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
QD	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
QG	3,29	1,93	0,55	0,82	2,60	0,00	1,06	1,54	1,47	0,14
QI	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
QK	0,00	0,00	0,69	0,00	0,00	0,00	0,00	0,92	0,00	0,57
QL	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
QP	3,29	0,00	0,83	1,97	2,49	0,00	0,26	0,00	0,00	0,00
QQ	0,49	0,00	0,00	0,33	0,22	0,00	0,00	0,00	0,00	0,00
QR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00
QS	0,16	0,00	0,41	0,00	0,00	0,00	0,00	0,00	0,00	0,00
QT	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,57
QV	0,00	0,00	0,00	0,74	0,00	0,00	0,00	0,00	0,74	0,43
QW	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
QY	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
RA	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
RC	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
RD	0,00	0,00	1,51	0,00	0,00	0,00	0,00	1,23	0,25	0,00
RE	0,99	0,77	0,00	0,08	0,00	0,00	0,53	0,00	0,00	0,00
RF	0,00	0,00	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
RG	1,32	4,12	2,61	2,38	2,60	0,00	1,06	1,54	2,95	1,28
RK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
RL	0,16	0,00	0,28	0,25	0,00	0,00	0,00	0,00	0,00	0,28
RM	0,16	0,00	0,00	0,41	0,00	0,00	0,00	0,00	0,98	0,00
RN	0,00	0,00	0,83	0,00	0,00	0,00	0,00	0,31	0,00	0,00

RP	0,99	0,51	0,96	1,97	0,00	0,00	0,79	0,00	1,23	0,57
RQ	0,00	0,00	0,83	0,08	0,00	1,62	0,00	0,00	0,00	0,14
RR	0,82	0,00	0,00	0,99	0,00	0,00	0,00	0,00	0,00	0,43
RS	0,00	0,00	0,41	0,90	0,00	0,00	0,00	0,00	0,00	0,71
RT	0,00	0,00	0,96	0,08	0,00	0,00	0,00	0,00	0,00	0,00
RV	0,00	0,00	0,00	0,33	0,00	0,00	0,00	0,00	0,49	0,00
RW	0,99	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SA	0,82	0,90	0,00	0,82	0,00	0,00	1,06	0,00	0,25	0,14
SD	0,00	0,00	0,00	0,00	1,08	0,00	1,06	0,00	0,00	0,00
SE	0,99	0,00	0,69	0,00	0,00	1,08	0,00	0,00	0,00	0,14
SF	0,33	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,43
SG	0,16	0,00	0,00	0,25	0,00	0,00	4,49	2,46	0,00	0,99
SI	0,00	0,00	0,28	0,08	0,00	0,00	1,06	1,23	0,00	0,00
SK	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
SL	0,99	0,00	0,83	1,23	0,11	0,00	0,00	0,00	0,00	0,00
SM	0,00	0,00	0,00	0,00	0,54	0,00	0,00	0,00	0,00	0,71
SN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	0,00
SP	0,99	0,00	0,55	0,08	0,65	0,00	0,00	0,00	0,00	0,85
SQ	0,00	0,39	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
SR	0,00	0,00	0,00	0,25	0,00	0,00	0,00	0,31	0,00	0,00
SS	0,00	0,90	0,00	0,66	0,11	0,00	0,00	0,00	0,00	0,00
SV	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
SW	0,00	1,54	0,83	0,00	0,00	0,00	1,06	1,23	0,98	1,56
SY	0,00	0,90	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TA	0,00	0,00	0,00	0,08	0,76	0,00	0,26	0,00	0,74	0,00
TC	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
TD	0,00	0,00	0,83	0,16	0,00	0,00	0,00	0,00	0,00	0,00
TE	0,16	0,00	0,83	0,16	0,00	0,00	0,00	0,00	0,00	0,00
TG	0,49	0,90	0,00	0,00	0,43	0,00	0,53	0,00	1,23	0,71
TI	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TK	0,00	0,00	0,00	1,48	0,54	0,00	0,00	0,00	0,00	0,00
TL	0,00	0,00	0,00	0,58	0,00	0,00	0,00	0,00	0,00	0,00
TP	0,16	0,00	0,00	1,07	0,54	0,00	2,64	2,46	0,00	0,14
TQ	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

TR	0,00	0,00	0,00	0,00	0,87	0,00	0,00	0,00	0,74	0,00
TS	0,00	0,00	0,00	0,66	0,00	0,00	0,00	0,00	0,00	0,43
TT	0,00	0,00	0,00	0,00	0,00	0,00	0,79	0,00	0,98	0,00
TV	1,15	0,00	0,00	0,66	1,73	0,00	0,00	0,00	0,00	0,00
TW	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
TY	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VA	0,00	0,00	0,96	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VD	0,00	0,00	0,00	0,08	0,65	0,00	0,00	0,00	0,98	0,57
VE	0,00	0,77	0,00	0,41	0,65	0,00	0,00	0,00	0,00	0,28
VF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00
VG	0,00	0,00	0,83	0,25	0,22	0,00	0,79	0,00	0,00	1,28
VH	0,00	0,00	0,69	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VI	0,99	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
VK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,43
VL	0,00	0,00	0,00	0,00	0,65	0,00	1,06	0,00	0,00	0,00
VM	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
VN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,62	0,00	0,00
VP	1,15	1,67	0,00	1,23	0,00	2,16	0,00	1,54	0,00	0,71
VQ	0,00	0,00	0,28	0,08	0,00	0,00	0,00	0,00	0,00	0,00
VR	0,00	0,00	0,00	0,25	0,65	0,00	0,00	0,00	0,00	0,00
VS	0,00	0,00	0,28	0,41	0,22	0,00	0,00	1,23	0,00	0,57
VT	0,00	0,00	0,00	0,00	0,76	0,00	2,11	0,00	0,00	0,00
VV	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VW	0,00	0,00	0,00	0,41	0,00	0,00	0,00	1,23	0,00	0,57
VY	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00
WE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00
WS	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
YA	0,00	0,00	0,28	0,16	0,00	0,00	0,00	0,00	0,00	0,00
YD	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
YE	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
YG	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,25	0,00
YI	0,00	0,00	0,00	0,00	0,76	0,00	0,00	0,00	0,00	0,00
YK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00
YP	0,16	0,00	0,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00

YQ	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00
YR	0,99	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Supplemental Table 10: Cleavage site frequency for C-terminal of identified NSGP.

%	T1D 1	T1D 2	T1D 3	T1D 4	T1D 5	Ctrl 1	Ctrl 2	Ctrl 3	Ctrl 4	Ctrl 5
AA	1,16	2,57	0,41	0,16	0,11	0,00	0,00	0,00	0,00	1,84
AG	2,16	1,80	0,83	3,37	1,08	7,57	0,00	1,23	0,98	0,71
AH	0,00	0,00	1,38	0,00	0,00	0,00	0,00	0,00	0,00	0,00
AK	0,00	0,77	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
AL	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
AP	5,80	4,63	2,61	4,44	3,25	2,16	5,48	3,38	4,91	4,11
AQ	0,00	0,00	0,00	0,00	0,00	1,62	1,04	1,23	0,00	0,00
AR	0,17	0,00	0,00	0,00	0,00	0,00	1,31	1,23	0,00	0,14
AS	0,33	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
AV	0,00	0,00	0,41	0,00	0,43	0,00	0,00	0,00	0,00	0,14
CG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
CK	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CP	0,00	0,00	0,00	0,33	0,22	1,62	0,00	0,00	0,00	0,28
DA	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
DE	0,00	0,00	0,55	0,00	0,00	0,00	0,00	0,00	0,00	0,14
DG	0,00	0,90	0,00	0,49	0,00	0,00	0,00	0,00	3,19	1,70
DI	0,17	0,00	3,58	1,73	1,73	1,08	0,00	0,00	0,00	0,00
DK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,71
DL	0,00	0,00	0,00	0,00	0,00	0,00	0,26	0,00	0,98	0,00
DN	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,14
DP	1,00	0,90	0,00	0,00	0,00	1,62	1,31	1,23	0,49	1,84
DQ	0,00	0,00	0,28	0,00	0,00	1,62	0,00	0,00	0,00	0,57
DR	0,00	0,77	0,00	0,00	0,11	0,00	0,00	0,00	1,72	0,00
DT	0,00	0,00	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
DV	0,17	0,00	1,10	0,49	0,00	0,00	0,00	0,00	0,98	0,00
EA	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
EE	0,33	0,00	0,83	0,58	0,00	0,00	0,00	0,00	0,00	0,14
EG	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EH	0,00	0,00	0,28	0,08	0,00	0,00	0,00	0,00	0,00	0,00
EI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
EK	0,00	0,00	0,69	0,00	0,00	0,00	0,00	0,00	0,00	1,70

EL	0,00	0,00	0,00	0,00	0,11	0,00	0,78	0,92	0,00	0,00
EN	0,00	1,54	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
EP	2,65	3,86	7,57	3,87	5,95	0,00	3,39	1,23	5,90	1,28
EQ	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,43
ER	0,00	0,00	0,96	0,00	0,00	0,00	0,00	0,00	0,49	0,00
EV	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,13
EW	0,00	0,00	1,38	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FG	0,00	0,00	1,38	0,00	0,00	0,00	0,00	0,00	0,00	0,57
FH	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
FI	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FK	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
FN	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FP	1,16	1,54	1,38	1,23	2,92	0,00	2,61	1,54	1,97	0,28
FR	0,00	0,00	0,00	0,00	0,00	2,70	0,00	0,00	0,00	0,00
FT	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GA	1,82	0,00	0,00	0,00	0,00	0,00	0,78	0,92	0,00	0,00
GD	0,00	0,00	0,96	0,00	0,00	0,00	0,00	0,00	0,49	0,00
GF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00
GG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,71
GH	0,00	0,00	1,24	0,16	0,00	0,00	0,00	0,00	0,00	0,00
GI	0,00	0,00	0,00	0,00	0,76	0,00	0,00	0,00	0,00	0,00
GK	0,00	0,00	0,00	0,91	0,00	2,70	0,00	1,54	0,98	0,00
GL	1,49	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GN	0,00	0,00	0,00	0,00	0,00	0,00	1,04	0,00	0,49	0,28
GP	15,42	24,97	13,34	13,17	15,80	13,51	22,45	18,15	12,53	18,58
GQ	1,66	0,00	0,00	0,16	0,00	0,00	1,31	0,92	0,00	0,00
GR	2,16	0,00	1,79	0,00	0,65	0,00	0,52	1,23	0,00	0,00
GT	0,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	0,57
GV	0,00	0,00	0,00	0,16	0,87	0,00	0,00	0,00	0,00	0,99
GY	0,00	0,00	0,00	0,00	0,00	0,00	1,04	0,00	0,00	0,00
HF	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
HI	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
HP	0,00	0,51	0,28	1,07	1,73	4,86	1,57	0,92	0,00	1,70
HQ	0,00	0,00	0,00	0,00	0,00	2,16	0,00	0,00	0,00	0,00

HV	1,00	0,00	0,00	0,99	0,00	0,00	0,00	0,00	0,00	1,13
IE	0,00	0,00	0,00	0,00	0,22	0,00	0,00	0,00	0,00	0,00
IG	1,00	0,00	0,00	0,08	0,00	2,70	0,00	0,00	0,74	0,14
II	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IK	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IL	0,00	0,00	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
IN	0,00	0,00	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IP	0,17	0,77	0,00	1,81	1,73	0,00	2,61	2,46	0,00	1,56
IQ	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,00
IV	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
KE	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
KG	1,16	0,39	0,00	0,00	0,00	2,70	0,00	0,00	0,00	0,00
KH	0,00	0,00	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00
KI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,98	0,00
KK	1,00	0,00	0,00	0,00	0,00	0,00	1,04	0,00	0,00	0,00
KL	0,00	0,00	0,00	0,08	0,00	0,00	1,04	0,00	0,00	0,00
KN	0,00	0,00	0,00	0,74	0,00	0,00	0,00	0,00	0,00	0,00
KP	1,49	0,77	1,38	2,55	1,84	0,00	3,13	3,69	1,97	2,13
KR	0,00	0,00	0,69	0,00	0,00	0,00	0,00	0,00	0,00	0,00
KS	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00
KV	0,00	0,00	0,00	0,82	1,30	0,00	0,00	2,46	0,74	0,14
LG	0,00	0,64	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
LH	0,00	0,00	0,69	0,74	0,00	0,00	0,00	0,00	0,00	0,00
LI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,00	0,00
LK	1,16	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,98	0,14
LL	0,50	0,64	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00
LN	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,31	0,00	0,00
LP	5,97	6,44	5,50	7,24	7,90	5,95	7,05	3,38	3,69	6,38
LR	0,50	0,00	0,00	0,00	0,00	0,00	0,78	0,00	0,00	0,00
LS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	0,00
LV	0,00	0,00	0,00	0,33	0,11	0,00	0,00	0,00	0,00	0,14
MD	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ME	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,43
MG	0,00	0,00	0,00	0,41	0,00	0,00	0,00	0,00	1,72	0,43

MK	0,00	0,64	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,00
MP	0,00	0,00	0,00	0,00	0,00	2,16	0,00	1,23	0,00	0,85
MQ	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
MR	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NG	0,00	0,00	0,00	0,00	0,00	2,70	0,00	0,00	0,00	0,00
NK	0,00	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00
NL	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,00	0,00
NN	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
NP	0,83	0,00	0,83	0,49	0,76	0,00	2,09	0,00	1,23	0,57
NQ	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
NS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
NV	0,00	0,77	0,00	0,16	0,22	0,00	0,00	0,00	0,00	0,00
PA	0,50	0,77	0,00	0,16	1,52	0,00	0,00	0,00	0,00	0,00
PD	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
PC	0,00	0,00	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,00
PE	0,00	0,00	0,00	0,00	0,65	0,00	0,00	1,23	0,00	0,00
PG	2,16	5,79	2,75	4,61	4,11	17,30	1,83	2,46	3,19	3,12
PH	0,17	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,14
PI	1,00	0,00	0,00	0,00	0,00	1,62	0,00	0,00	0,00	0,43
PK	0,66	0,77	0,00	0,58	0,00	0,00	0,00	0,00	0,00	0,00
PL	0,66	0,00	0,69	0,08	0,43	0,00	0,00	0,00	0,00	0,00
PM	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PN	0,00	0,00	0,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00
PP	18,41	22,14	15,96	18,85	17,53	16,22	18,28	21,54	18,92	17,59
PQ	0,17	0,77	1,10	0,74	0,00	0,00	0,00	0,00	0,49	0,00
PR	0,83	0,00	0,96	0,08	0,76	0,00	0,00	0,92	0,00	0,71
PS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
PV	0,17	0,77	0,00	0,58	1,41	0,00	0,00	0,00	2,95	0,00
QA	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
QE	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
QG	0,66	0,77	2,48	0,33	2,49	1,08	3,13	0,00	0,00	0,71
QI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,28
QK	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
QL	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00

QP	2,65	2,83	2,06	2,63	3,79	0,00	0,78	0,00	3,93	0,43
QQ	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,74	0,00
QR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
QS	0,00	0,00	0,41	0,16	0,00	0,00	0,00	0,00	0,00	0,00
QV	0,00	0,00	0,00	0,08	0,54	0,00	0,00	0,00	0,00	0,00
RA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,43
RF	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
RG	0,00	0,00	0,96	0,49	1,08	1,62	0,00	0,00	0,00	0,99
RK	0,00	0,00	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,43
RN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,74	0,00
RP	1,00	1,80	1,79	1,65	1,08	0,00	1,04	1,54	1,23	0,85
RR	0,00	0,00	0,55	0,00	0,00	0,00	0,00	0,00	0,00	0,28
RS	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,14
SA	1,16	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,57
SF	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,43
SG	0,17	0,00	0,00	0,66	0,00	0,00	0,52	0,92	0,00	0,14
SL	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,31	0,00	0,00
SN	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,43
SP	4,64	1,67	0,96	2,63	3,68	2,70	0,26	0,00	1,47	2,13
SQ	0,00	0,00	0,00	0,00	0,11	0,00	0,00	0,00	0,00	0,28
SR	0,83	0,00	0,00	0,08	0,11	0,00	0,00	0,00	0,00	0,14
ST	0,00	0,00	0,00	0,00	0,00	0,00	1,04	0,00	0,00	0,00
SV	0,00	0,00	0,00	0,58	0,00	0,00	0,00	0,00	0,98	0,00
SW	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TG	0,66	0,00	0,69	1,73	0,54	0,00	0,00	0,92	2,46	0,99
TI	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,57
TK	0,00	0,00	0,00	0,25	0,54	0,00	0,26	0,00	0,00	0,00
TL	1,00	0,77	0,00	0,00	0,00	0,00	2,09	0,00	0,00	0,00
TP	0,17	0,00	3,16	3,13	3,03	0,00	2,61	3,69	0,00	1,84
TQ	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,14
TS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,57
TV	0,00	0,77	0,28	0,58	0,11	0,00	0,00	0,00	0,00	0,57
VA	0,17	0,00	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00

VF	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00
VG	0,00	0,77	0,83	0,00	0,00	0,00	0,52	0,92	0,00	1,13
VH	0,00	0,00	0,83	0,08	0,00	0,00	0,00	0,00	0,00	0,00
VI	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00
VL	0,00	0,00	0,00	0,66	0,00	0,00	0,00	0,00	0,00	0,00
VN	0,00	0,00	0,83	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VP	2,16	0,90	4,68	4,69	1,84	0,00	2,87	5,23	10,57	4,82
VQ	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
VR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00
VV	0,00	0,00	0,28	0,00	0,00	0,00	0,00	0,00	0,74	0,28
WK	0,00	0,00	0,00	0,00	0,11	0,00	1,31	0,00	0,00	0,00
WP	0,66	0,00	0,00	0,08	0,00	0,00	0,78	0,00	0,00	0,00
YG	0,00	0,51	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
YN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,23	0,00	0,00
YP	0,00	0,00	0,28	0,49	0,00	0,00	0,00	1,54	0,25	1,56
YV	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Supplemental Table 11: Contingency table for N-terminal of identified SGP in controls.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A						0,23	0,10						0,00	0,25	0,21						0,79
C																					
D						0,17					0,07										0,24
E						7,17									0,77	0,33					8,27
F						2,03	0,12	0,21					0,10					0,80		0,43	3,69
G					1,28			0,31	0,80	0,26		0,07	1,56	0,26	0,69					0,08	5,31
H	0,03													0,07	0,97						1,07
I																					
K	0,12	0,03		0,03	0,03		0,09						1,86		0,09	0,16					2,41
L	0,44					0,06														0,24	0,74
M																					
N									0,13			0,13	0,17	0,13							0,56
P	0,57				0,15	0,17	0,07			0,28			3,62	3,46	4,53	0,43				0,65	13,93
Q	0,77			0,30		44,20							0,34	0,88	0,21	1,73				0,39	48,82
R	0,20		0,09	0,08		0,68		1,24	0,12				0,17	0,10	0,13	2,17					4,98
S	0,68					0,03			0,07				0,24	0,25	1,84						3,11
T					0,06																0,06
V																					
W																					
Y						5,39								0,15	0,46		0,03				6,03
Total	2,81	0,03	0,09	0,41	1,52	60,13	0,38	1,76	1,12	0,54	0,07	0,20	8,06	5,55	9,90	4,82	0,03	0,80		1,79	100

The amino acids are represented in one letter code

Supplemental Table 12: Contingency table for C-terminal of identified SGP in controls.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A			0,32			0,60			0,24				0,54			0,08					1,78
C																					0,00
D				0,32		0,64															0,96
E				0,12		0,10								0,10	0,35	0,13					0,80
F						1,24	0,24	0,23		0,02								0,87		0,03	2,63
G					0,73	0,85	0,15		0,21				2,45		1,11					0,16	5,66
H				0,11			0,05								0,22	0,05					0,43
I						0,08								0,03							0,11
K	0,16				0,32		0,21					0,06	2,00		0,02	0,60					3,37
L	0,76				0,24			0,13				0,06						0,02		0,17	1,38
M										0,03											0,03
N													0,45								0,45
P				0,03		1,37							5,70	2,20	1,46	0,50		0,11		0,57	11,94
Q	1,01		0,13		0,30	43,09							0,56	1,49	0,12	6,58				0,05	53,33
R	0,23		0,05		0,02	3,17	0,08	0,74	0,24	0,11			1,47	0,15		3,53					9,79
S			0,30				0,05						1,15	1,92				0,03			3,45
T																					0,00
V													0,14								0,14
W																					0,00
Y						3,14							0,50	0,11							3,75
Total	2,16	0,00	0,80	0,58	1,61	54,28	0,78	1,10	0,69	0,16	0,00	0,12	14,96	6,00	3,28	11,47	0,00	1,03	0,00	0,98	100

The amino acids are represented in one letter code

Supplemental Table 13: Contingency table for N-terminal of identified SGP in diabetic patients.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A						0,07	0,22						0,02	0,26	0,15	0,01					0,73
C																					0,00
D										0,01											0,01
E						6,55			0,05					0,10	0,51	0,26					7,47
F						1,91	0,04	0,55										0,34		3,88	6,72
G					1,37	0,66		0,23	0,61	0,28		0,12	1,62	0,05	0,33					0,75	6,02
H							0,34						0,05		1,37	0,88					2,64
I																					0,00
K	0,05				0,26		0,05			0,02			0,90		0,49	0,09					1,86
L	0,71					0,12							0,09							1,54	2,46
M													0,02								0,02
N									0,09					0,15						0,18	0,42
P	0,38				0,27	0,98	0,12	0,04		0,09	0,02		5,02	2,42	3,13	0,18				0,63	13,28
Q	0,61			0,21		33,32		0,05		0,04			0,10	0,87	0,41	1,74				2,04	39,39
R	0,03		0,03	0,14		0,31	0,04	0,39	0,32				0,15		0,14	1,62					3,17
S	0,96			0,02	0,04		0,13		0,12			0,80		0,21	0,79	0,11		0,02			3,20
T					0,28					0,04			0,04								0,36
V					0,04											0,02					0,06
W																					0,00
Y			0,53			8,03			0,31	0,25			0,04	1,47	1,48		0,09				12,20
Total	2,74		0,56	0,37	2,26	51,95	0,94	1,26	1,50	0,73	0,02	0,92	8,05	5,53	8,80	4,91	0,09	0,36	0,00	9,02	100

The amino acids are represented in one letter code

Supplemental Table 14: Contingency table for C-terminal of identified SGP in diabetic patients.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A			0,68			0,23			0,37				0,51			0,03					1,82
C																					0,00
D				0,16		0,37									0,05	0,32					0,90
E				0,16					0,73	0,03				0,15	0,16						1,23
F				0,11		1,66	1,58	0,30		0,26			0,35					0,25		0,36	4,87
G					0,59	0,63			0,25	0,03		0,05	1,24		1,00					0,39	4,18
H				0,90	0,16		1,17						0,17		0,50	1,08				0,03	4,01
I			0,24			0,22							0,08	0,01							0,55
K	0,06				1,85		2,00					0,05	1,67		0,16	0,55					6,34
L	0,58							0,05				0,07	0,10			0,09				0,89	1,78
M																	0,03				0,03
N													0,09	0,05							0,14
P						1,00	0,08						6,35	1,48	1,11	0,44		0,25		0,70	11,41
Q	0,47		0,21	0,01	0,34	32,72				0,01			0,51	0,71	0,19	5,24				0,33	40,74
R	0,13		0,03	0,07		2,86	0,25	1,97	1,62	0,04			0,97	0,02	0,60	3,62					12,18
S	0,16		0,43			0,51	0,83						0,95	1,43		0,13	0,04	0,02			4,50
T													0,07								0,07
V													0,48								0,48
W																					0,00
Y						3,97			0,03				0,21	0,51							4,72
Total	1,40	0,00	1,59	1,41	2,94	44,17	5,91	2,32	3,00	0,37	0,00	0,17	13,75	4,36	3,77	11,50	0,07	0,52	0,00	2,70	100

The amino acids are represented in one letter code

Supplemental Table 15: Contingency table for N-terminal of identified NSGP in controls.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A	0,74	0,35	0,31	0,15		1,23		0,26	0,15			0,05	0,58	0,25	0,70	0,16	0,71	0,31			5,95
C	0,10		0,06				0,11					0,25									0,52
D			0,11			0,76		0,11	0,06		0,09		0,73	0,36	0,09			0,21			2,52
E	0,84		0,20	0,97	0,05	0,30			0,34	0,46		0,21	1,37	0,11	0,03	0,26	0,51	0,44	0,21		6,30
F				0,31					0,06	0,13		0,46	0,71	0,25			0,79	0,11			2,82
G	0,94		0,33	1,28	1,09	0,20		0,23	0,10	2,02	0,26	0,24	8,70	0,58	0,81	0,57	0,45	1,32		0,20	19,32
H						0,19	0,03			0,11	0,57		0,30		0,14	0,05					1,39
I	0,25				0,44	0,89			0,18		0,15		0,33		0,21	0,14	0,17				2,76
K			0,25			2,33			0,51				2,19	1,01		0,32	0,16	0,70			7,47
L	0,72				0,25	0,88	0,03	0,34	0,11	0,27		0,65	2,70	0,60	1,57	0,40		0,05			8,57
M	0,03		0,18			0,34					0,16		0,03								0,74
N		0,03	0,31		0,05	0,06					0,11		0,09			0,12					0,77
P	0,75	0,18	0,11	1,23	0,21	10,76		0,53	1,12	0,44	0,79		4,19	0,56	1,50	0,36	0,53	1,76			25,02
Q	0,03					0,84			0,30				0,05		0,05		0,11	0,23			1,61
R			0,30	0,11		1,37			0,03	0,06	0,20	0,06	0,52	0,35	0,09	0,14		0,10			3,33
S	0,29		0,21	0,24	0,09	1,59		0,46			0,14	0,20	0,17		0,06			0,03	0,97		4,45
T	0,20					0,49							1,05		0,15	0,09	0,35		0,11		2,44
V			0,31	0,06	0,05	0,41		0,25	0,09	0,21		0,12	0,88			0,36	0,42		0,36		3,52
W				0,10																	0,10
Y			0,11			0,05			0,10					0,25							0,51
Total	4,89	0,56	2,79	4,45	2,23	22,69	0,17	2,18	3,15	3,70	2,47	2,24	24,59	4,32	5,40	2,97	4,20	5,26	1,65	0,20	100

The amino acids are represented in one letter code

Supplemental Table 16: Contingency table for C-terminal of identified NSGP in controls.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A	0,37					2,10			0,25				4,01	0,78	0,54			0,03			8,08
C						0,25							0,38								0,63
D				0,03		0,98		0,22	0,39	0,25		0,03	1,30	0,44	0,34			0,20			4,18
E				0,03				0,25	0,34	0,34			2,36	0,09	0,10			0,23			3,74
F						0,11			0,11				1,28		0,54						2,04
G	0,34		0,10		0,10	0,14			1,04			0,36	17,04	0,45	0,35		0,31	0,20		0,21	20,64
H													1,81	0,43				0,23			2,47
I						0,72							1,33								2,05
K						0,54		0,44	0,21	0,21			2,18					0,67			4,25
L								0,06	0,47			0,06	5,29		0,16	0,20		0,03			6,27
M				0,09		0,43							0,85								1,37
N						0,54				0,06			0,78			0,11					1,49
P			0,03	0,25		5,58	0,03	0,41					18,51	0,10	0,33	0,03		0,59			25,86
Q						0,98		0,06	0,03				1,03	0,15	0,11						2,36
R	0,09					0,52			0,09			0,21	0,93		0,06	0,03					1,93
S	0,11				0,09	0,32				0,06		0,09	1,31	0,06	0,03		0,21	0,20			2,48
T						0,87		0,11	0,05	0,42			1,63		0,13	0,11		0,11			3,43
V						0,51							4,70		0,05			0,20			5,46
W									0,26				0,16								0,42
Y												0,25	0,67								0,92
Total	0,91	0,00	0,13	0,40	0,19	14,59	0,03	1,55	3,24	1,34	0,00	1,00	67,55	2,50	2,74	0,48	0,52	2,69	0,00	0,21	100

The amino acids are represented in one letter code

Supplemental Table 17: Contingency table for N-terminal of identified NSGP in diabetic patients.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A	0,60	0,17	0,70	0,27		2,40		0,40	0,36	0,39	0,02	0,03	1,42	0,59	0,11	0,32	0,18	0,43		0,02	8,41
C								0,02		0,18						0,02	0,03				0,25
D			0,10			1,51		0,10	0,24	0,02	0,10		0,18		0,57	0,10		0,20		0,15	3,27
E	0,18		0,26	0,05	0,10			0,20	0,89				0,95	0,10		0,36	0,14	0,29			3,52
F			0,10	0,23		0,07		0,40				0,03	0,45		0,13	0,03		0,13			1,57
G	3,34	0,07	1,18	1,87	1,70	0,29		0,46	0,75	1,53	0,15	0,46	6,03	1,48	1,28	0,73	0,29	1,24	0,02	0,20	23,07
H		0,13				0,04	0,02	0,26	0,12	0,10			0,09	0,12		0,13	0,02				1,03
I	0,10			0,30	0,05	0,45			0,19	0,13	0,22		0,16	0,18	0,17	0,10	0,20			0,25	2,50
K	0,61	0,03	0,04	0,14		2,74				0,02	0,14		0,58	0,07		0,27	0,32	0,62			5,58
L	0,17	0,10	0,14	0,16	0,02	0,28	0,02	0,03	0,96	0,19		0,33	0,84	0,03	0,37		0,28	0,27		0,06	4,25
M	0,15					0,63				0,13	0,09			0,15							1,15
N	0,15		0,03						0,03	0,17			0,19			0,18	0,02				0,77
P	1,15	0,02		0,87		9,73	0,25	0,15	0,77	0,50	0,04		4,96	0,59	0,77	1,91	1,10	0,95		0,24	24,00
Q	0,12	0,02	0,03			1,84		0,02	0,14	0,07			1,72	0,21		0,11	0,10	0,15	0,02	0,10	4,65
R	0,15	0,02	0,30	0,37	0,18	2,61				0,14	0,11	0,17	0,89	0,18	0,36	0,26	0,21	0,07	0,20		6,22
S	0,51		0,22	0,34	0,08	0,08		0,07	0,10	0,63	0,11		0,45	0,09	0,05	0,33			0,47	0,18	3,71
T	0,17	0,02	0,20	0,23		0,36		0,06	0,40	0,12			0,35	0,03	0,17	0,13	0,00	0,71		0,03	2,98
V	0,19		0,15	0,37		0,26	0,14	0,20		0,13	0,13		0,81	0,07	0,18	0,18	0,15	0,06	0,08	0,06	3,16
W																0,03					0,03
Y	0,09		0,15	0,02		0,06		0,15					0,10		0,20						0,77
Total	7,68	0,58	3,60	5,22	2,13	23,35	0,43	2,52	4,95	4,45	1,11	1,02	20,17	3,89	4,36	5,19	3,04	5,12	0,79	1,29	100

The amino acids are represented in one letter code

Supplemental Table 18: Contingency table for C-terminal of identified NSGP in diabetic patients.

%	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Total
A	0,88					1,85	0,28		0,15	0,03			4,15		0,03	0,20		0,17			7,74
C									0,15				0,11								0,26
D	0,17			0,11		0,28		1,44				0,03	0,38	0,06	0,18		0,18	0,35			3,18
E	0,02			0,35		0,15	0,07		0,14	0,02		0,49	4,78	0,06	0,19			0,15	0,28		6,70
F						0,28	0,02	0,03				0,06	1,65				0,03				2,07
G	0,36		0,19				0,28	0,15	0,18	0,30			16,54	0,36	0,92		0,13	0,21			19,62
H					0,10			0,02					0,72					0,40			1,24
I				0,04		0,22		0,03	0,07	0,18		0,17	0,90	0,02				0,02			1,65
K				0,02		0,31	0,17		0,20	0,02		0,15	1,61		0,14	0,06		0,42			3,10
L						0,14	0,29		0,23	0,29		0,02	6,61		0,10			0,09			7,77
M			0,20			0,08			0,18					0,03	0,07						0,56
N									0,10			0,13	0,58	0,02				0,23			1,06
P	0,59	0,05		0,13		3,88	0,09	0,20	0,40	0,37	0,03	0,07	18,58	0,56	0,53			0,59			26,07
Q	0,13			0,02		1,35			0,17	0,06			2,79			0,11		0,12			4,75
R					0,03	0,51			0,05				1,46		0,11	0,02					2,18
S	0,36					0,17						0,13	2,72	0,02	0,20			0,12	0,03		3,75
T						0,72		0,03	0,16	0,35			1,90	0,17				0,35			3,68
V	0,13				0,02	0,32	0,18	0,13		0,13		0,17	2,85	0,20				0,06			4,19
W									0,02				0,15								0,17
Y						0,10							0,15					0,07			0,32
Total	2,64	0,05	0,39	0,67	0,15	10,36	1,38	2,03	2,20	1,75	0,03	1,42	68,63	1,50	2,47	0,39	0,34	3,35	0,31	2,64	100

The amino acids are represented in one letter code

Supplemental Table 19: MS/MS data of cathepsin D identification.

Start Sequence Position	End Sequence Position	Peptide sequence	Observed Mass	Calculated Mass	Match Error Da	Match Error PPM	Mascot score	C.I. %
393	404	YYTVFDRDNNRV	1561,69	1561,67	0,02	-12,81	45	100
153	172	QDTVSVPCQSASSASALGGV	1864,04	1864,01	0,03	-16,09	32	99
331	345	VSTLPAITLKLGGK	1397,86	1397,89	0,03	19,60	35	99
34	49	RTMSEVGGSVEDLIAK	1691,88	1691,92	0,04	21,69	44	100

C.I. – Confidence interval; Mascot score refers to individual peptide score from MS/MS data.

STUDY II - PROTEASE PROFILING OF DIFFERENT BIOFLUIDS IN TYPE 1 DIABETES MELLITUS

Supplementary table 1: Demographic and clinical characteristics of subjects.

	Group A	Group B	Group C	Group D
Age (years)	41.31±11.16	46.07±12.28	38.94±13.86	44.75±4.66
Gender (M/F sex ratio)	4/1	3/2	3/2	3/2
Duration of diabetes (years)	29.80±8.80	31.40±11.64	17.40±5.38	--------
Duration of retinopathy (years)	12.6±10.78	7.80±6.14	----------	---------
Duration of nephropathy (years)	4.05±3.35	----------	----------	---------
HbA1c (%)	8.92±1.61	8.18±0.60	7.71±0.09	5.17±0.42

The mean value of each characteristic is presented together with its corresponding standard deviation.

STUDY III - SALIVARY PROTEOME AND PEPTIDOME PROFILING IN TYPE 1 DIABETES MELLITUS USING A QUANTITATIVE APPROACH

Supplementary table S1: Proteins differentially regulated between T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl (Protscore refers to ProteinPilot score).

Protscore	% Coverage	Accession	Gene ID	Name	Peptides (95%)	T1D-R+N /Ctrl	*p* value	T1D-R /Ctrl	*p* value	T1D /Ctrl	*p* value
9,16	15.9	P01023	A2MG_HUMAN	Alpha-2-macroglobulin	6	3.1108	0.0098				
127,62	93	P04745	AMY1_HUMAN	Alpha-amylase 1	362					1.8948	0.0000
11.26	20.5	Q6ZME0	Q6ZME0_HUMAN	Bactericidal/permeability-increasing protein-like 1	6	1.3569	0.0207	1.4129	0.0176	1.7707	0.0359
3,2	26.6	Q6EZE9	Q6EZE9_HUMAN	Defensin, alpha 3, neutrophil-specific	2	1.6206	0.0071			0.4982	0.0026
15,04	31.6	Q9UGM3	DMBT1_HUMAN	Deleted in malignant brain tumors 1 protein	13			0.6656	0.0010	0.6247	0.0090
2,2	6.9	Q2TSD0	Q2TSD0_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase	3			1.7718	0.0496		
7.97	32.0	Q6GMX6	Q6GMX6_HUMAN	IGH@ protein	7			1.3964	0.0497		
6.45	17.9	KLK1	KLK1_HUMAN	Kallikrein-1	6			0.4763	0.0159		
15,04	44	P22079	PERL_HUMAN	Lactoperoxidase	12					1.6242	0.0009
2.37	17.9	P30740	ILEU_HUMAN	Leukocyte elastase inhibitor	6	1.3157	0.0472				
5,49	8.6	Q59GX5	Q59GX5_HUMAN	L-plastin variant (Fragment)	4	1.8157	0.0036	1.4002	0.0331		
6,38	37.8	B2R4C5	B2R4C5_HUMAN	Lysozyme C	5					5.4841	0.0341
2,16	14.9	P14780	MMP9_HUMAN	Matrix metalloproteinase-9	3	1.7854	0.0250				
24,4	22.7	Q9HC84	MUC5B_HUMAN	Mucin-5B	17					1.3125	0.0129
5,98	29.2	Q8TAX7	MUC7_HUMAN	Mucin-7	5					3.8097	0.0081
6,17	39	P08246	ELNE_HUMAN	Neutrophil elastase	7	1.5345	0.0386				
7,11	49	C3PTT6	C3PTT6_HUMAN	Pancreatic adenocarcinoma upregulated factor	14	1.7223	0.0041	1.2514	0.0169	2.3395	0.0009
3,8	14.8	P13796	PLSL_HUMAN	Plastin-2	4	1.8379	0.0038	1.3734	0.0363		
12	56.2	P12273	PIP_HUMAN	Prolactin-inducible protein	14					2.5702	0.0263
25,22	78.5	P05109	S10A8_HUMAN	Protein S100-A8	19	1.9484	0.0007	1.7727	0.0004		
17,28	95.6	P06702	S10A9_HUMAN	Protein S100-A9	24	1.5670	0.0143	1.4814	0.0064		
37,05	60.8	P02768	ALBU_HUMAN	Serum albumin	54	1.3098	0.0000				
25,83	49.1	Q6P5S2	CF058_HUMAN	UPF0762 protein C6orf58	28					2.1725	0.0328
20,83	55.7	P25311	ZA2G_HUMAN	Zinc-alpha-2-glycoprotein	20					1.7030	0.0026
10,01	59.6	Q96DA0	ZG16B_HUMAN	Zymogen granule protein 16 homolog B	18	1.7233	0.0003			2.0279	0.0005

Supplementary table S2: List of all salivary peptides identified using LC-MS/MS.

Conf	Protein Name	Accession Number	Sequence	Modifications	ΔMass	Prec MW
98,2	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNKPQGPPPPGKPQ		0,00	1454,76
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQ	Deamidated(N)@2; Pro->Asp@3	0,00	2433,16
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,19	4160,21
98,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPA		-0,01	1676,82
98,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQGPPPPGKPQ	Gln->Pro@5	-0,01	1959,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPAQGGSKSQ		0,01	2925,50
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	AGNPQGPSPQGGNKPQ	Ser->Pro@8	0,00	1542,75
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GKPQGPPPQGGNQPQ		-0,02	1485,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	ESPSLIAGNPQGPSPQGGNKPQGPPPPPGKPQ		0,03	3111,60
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPPRPPQGGRPS		0,00	1948,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQ		-0,16	1839,72
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNQPQGPPPPPGKPQ		0,00	1551,78
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPP		0,01	1015,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNQPQGPPPPPGKPQGPPPQGGNKPQ		0,01	2609,32
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQ		0,06	3148,60
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQ		0,00	1461,70
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	ESPSLIAGNPQGPSPQGGNKPQ		-0,01	2159,05
96,1	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPQGGRPSRPP		-0,02	1144,59
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQ	Lys->Gln@23	0,04	2414,21
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQ	Ala->Ser@15	0,06	3164,60
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	ESPSLIAGNPQGPSPQGGNKPQGPPPPPGKPQ	Deamidated(N)@9	0,02	3112,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQGPPPQGGNKPQ		0,02	3471,76
96,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQ		0,08	3963,00
96,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPP		0,00	1318,71
95,5	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@30	-0,01	3471,77
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSAR		0,00	1326,66
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQ		0,00	2002,04
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQ		0,00	1349,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQ		0,00	1340,65
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNKPQGPPPPPGKPQGPPPQGGNKPQ		-0,01	2609,34
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPP		0,00	2338,15
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPP		0,04	2688,39

99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSQSPRSPPGKPQ		0,06	2896,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQGGSKSQSA		-0,04	2025,98
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	Deamidated(N)@9	0,04	2817,43
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQGGSKSQ		-0,39	1867,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSQSP		0,00	2049,02
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS		0,08	3270,72
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@21	-0,01	3085,57
98,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPGKPQGPPPQGGNQPQ		-0,15	1679,69
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQSPPGKPQ	Pro->Asp@4; Deamidated(Q)@5	-0,01	2059,98
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ		0,00	2883,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQG		0,04	2873,47
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSP		0,00	1221,61
98,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNQPQGPPPPPGKPQGPPQQ		0,01	2059,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQ		0,00	1349,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQ		0,10	4004,10
98,9	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKS	Gln->Arg@25	-0,03	2822,43
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQSPPGKPQ		0,00	2041,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQGGNKPQ	Lys->Arg@19	0,00	2056,06
96,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQ		0,00	1075,54
97,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQ	Pro->Ala@12	0,00	1323,69
96,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPRSPPGKPQGPPPQGGNQPQ		0,01	2107,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPR		0,00	1377,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSRSPQSPPGKPQ		0,02	2896,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQ		-0,01	1930,99
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSQSPR		0,01	1349,68
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQSPPGKPQGPPPQGGNQPQ		0,04	3098,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKSQS	Ser->Met@29; Deamidated(Q)@30; Phospho(S)@31	0,15	3134,62
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPPAGGNPQQPQAPPAGQPQGPP		-0,01	2184,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQ		0,00	2028,01
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQ		0,03	2504,29
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Lys->Gln@30	0,07	3085,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPG		0,00	1674,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ	Deamidated(N)@29; Amidated@C-term	0,09	3941,10
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQ		0,01	1164,52
96,2	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSQSPR	Lys->Arg@17	0,03	2233,16
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQ		-0,01	2133,98

99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKSRS	Deamidated(Q)@25; Oxidation(R)@30; Phospho(S)@31	0,15	3134,64
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQ	Glu->Gln@6; Deamidated(N)@9	0,02	2487,17
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQ	Acetyl@N-term	-0,01	1117,51
97,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,01	3484,66
98,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQG		-0,04	1503,75
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPA		0,01	2823,34
95,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSP		0,02	3134,62
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPQQGGNRPQ		0,07	3144,63
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNKPQGPPPPPGKPQ		-0,04	1551,77
96,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNRPQGPPPPGKPQ		0,00	1482,77
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,07	3233,59
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQGPPPPGKPQ		0,00	1990,01
95,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPS		-0,03	1201,60
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@12	0,05	3777,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPSRPPQ		0,00	1679,90
96,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSRSPR		-0,06	2233,11
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ		0,07	3941,08
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GQPQGPPRPPQGGRPS		0,00	1611,83
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	Glu->Lys@6; Deamidated(N)@20	-0,08	3962,88
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQGGNKPQ		0,00	2028,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPQGGRPSRPPQ		0,00	1272,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQ		0,01	2087,03
97,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPQQ		0,00	1477,76
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QPQAPPAGQPQGPP		-0,01	1368,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPP		0,08	3834,94
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQ		0,01	1323,71
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPAQGGSKSQ	Lys->Gln@9	0,05	2925,50
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QPQAPPAGQPQGPPRPPQ		-0,01	1846,93
97,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNKPQGPPPPGKPQ		-0,02	1397,72
98,1	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQP	Asn->Lys@15	0,00	1652,87
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQ	Lys->Arg@9	0,00	1959,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQ		-0,16	1766,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQ		0,02	970,54
97,7	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ		0,09	3777,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQ	Deamidated(N)@2	0,02	2415,21
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQ	Lys->Gln@26	0,05	2719,39

99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQ		0,01	1446,77
98,5	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQ		0,05	3195,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPP	Deamidated(Q)@5	0,12	3835,96
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPQSPPGKPQGPPPQGGNQPQ		-0,01	2079,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPSRPPQ		0,08	3748,99
98,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPQQGGNRPQ		0,00	2087,07
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PSLIAGNPQGP		0,03	1049,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPG		0,00	2366,18
98,5	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQ	Pro->Asn@2	0,00	1856,87
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPQGDKSQSPRSPPGKPQ		0,00	1886,96
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PSPQGGNKPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ	Phospho(S)@2; Gln->Asp@4	0,13	3941,07
98,7	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQ		0,01	2487,16
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPQGDKSRSPQSPPGKPQ		0,00	1886,96
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGGNKPQGPPPPPGKPQ	Gln->pyro-Glu@N-term	0,00	1662,85
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGPPPQGGNKPQGPPPPGKPQ	Gln->Cys@1	0,02	2034,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQGPPPPGKPQGPPPQGDKS	Gln->Pro@5	0,05	2822,47
98,9	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQ	Ala->Ser@15	-0,02	2018,01
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPSRPP		0,00	1551,84
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QNLNEDVSQEESPSLIAGNPQGPSPQGGNKPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,08	3378,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQ		0,04	1075,55
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	RSPPGKPQGPPPQGGNQPQ	Phospho(S)@2; Oxidation(P)@4	0,09	2019,02
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SARAPPGKPQGPPQQEGNNPQ		0,01	2154,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	KPQGPPPQGGNKPQ		0,00	1428,75
97,9	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ		0,01	2816,42
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SLIAGNPQGPSPQGGNKPQ		0,00	1845,93
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQP	Asn->Gln@15	-0,02	1652,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQ		0,00	2414,21
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQ		0,02	2719,40
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQ	Lys->Glu@5	0,04	2720,37
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQG	Lys->Ala@37	0,16	3777,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PQGPPPPGKPQGPPPQ	Gln->Pro@2	0,00	1543,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGPPPPPGKPQGPPPQ	Gln->Pro@1	0,00	1543,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPQSPPGKPQGPPPQGGNQPQGPPPPPGKPQ		0,06	3031,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSQSPRSPPGKPQ		-0,01	2041,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Lys->Arg@37	0,09	3805,01
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPPRPPQ		0,00	1493,77

99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,11	3909,01
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSQSPRSPPGKPQGPPPQGGNQPQ		0,05	3098,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ	Glu->Lys@6; Deamidated(N)@9	0,01	3233,57
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKS		0,00	2794,42
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQ	Acetyl@N-term	-0,05	2069,98
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ		0,03	3085,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	Deamidated(Q)@25; Amidated@C-term	0,08	3962,99
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQ		0,01	1185,62
98,7	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS		0,14	4417,29
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	RPPQGGRPSRPPQ		0,00	1428,77
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@33	0,05	3777,00
98,1	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQ		0,01	1134,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQ		0,08	3162,60
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GGNKPQGPPSPPGKPQ		0,00	1541,79
98,5	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSR		0,02	1151,58
98,8	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSRSS		0,01	1325,64
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	AGNPQGAPPQGGNKPQ	Ala->Pro@7	0,00	1542,75
98,3	Basic salivary proline-rich protein 2	PRB2_HUMAN	QGGNKPQGPPSPPGKPQ	Asn->Pro@4; Deamidated(Q)@17	0,02	1653,86
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GNPQGAPPQGGNKPQGPPSPPGKPQ		0,00	2388,19
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	ARSPPGKPQGPPPQGGNQPQ	Arg->Met@2	-0,20	1968,75
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDNKS		0,01	2908,47
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQ		-0,14	1978,85
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQSA		-0,05	2137,00
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSARSPPGKPQGPPPQGGNQPQ		0,08	3186,63
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSARSPPGKPQ	Lys->Gln@19	0,03	2129,05
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSRSS		-0,14	2180,95
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSAR		0,00	1437,69
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSARSPPGKPQ		0,00	2129,06
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGGSKS		0,03	2823,47
95,2	Basic salivary proline-rich protein 2	PRB2_HUMAN	GNPQGAPPQGGNKPQ		0,00	1445,70
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDNK	Lys->Thr@29	0,03	2794,41
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKSQGPPPPGKPQ	HexNAc(N)@8	-0,01	2124,05
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSRSSRSPPGKPQ		0,00	2173,09
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGSKSRSS		0,00	1240,62
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPSPPGKPQGPPPQGGNQPQ	Lys->Glu@8	0,08	2019,02
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQ	Lys->Gln@5	0,07	3178,56

99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPSPPGKPQ		0,00	960,51
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	QNLNEDVSQEESPSLIAGNPQGAPPQGGNKPQ	Gln->pyro-Glu@N-term; Phospho(S)@8; Ala->Ser@23	0,07	3378,57
96,8	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQS	Gln->Tyr@20; Phospho(S)@21	-0,03	2180,96
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQ		-0,17	1855,71
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	QGGNKPQGPPSPPGKPQGPPPQGGNQPQ	Gln->pyro-Glu@N-term	0,08	2710,40
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQ		0,05	2825,40
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQ		0,06	3178,57
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,16	3925,05
97,1	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGGSKSR		-0,01	1922,01
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,15	4176,16
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SRSPPGKPQGPPPQGGNQPQ	Arg->Asp@2	0,00	1968,93
95,9	Basic salivary proline-rich protein 2	PRB2_HUMAN	SARSPPGKPQGPPQQEGNNPQ		-0,06	2169,99
97,4	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGSKSRSA		0,01	1224,63
96,1	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKS		-0,12	1850,80
98	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQE		0,01	1345,67
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GNPQGAPPQGGNKPQ	Oxidation(P)@7	0,00	1461,70
96,5	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSRSS	Lys->Gln@7	-0,16	2180,89
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GGNKSQGPPPPGKPQ	HexNAc(N)@3	0,00	1647,82
95,6	Basic salivary proline-rich protein 3	PRB3_HUMAN	SGKPEGRRPQGGNQPQ		0,00	1691,84
95,5	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPRPGKPEGSPSQGGNKPQ		-0,01	2283,13
98,3	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPE	Acetyl@N-term	-0,05	1268,56
95,4	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPHPGKPQ		0,01	1010,54
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGSPSQGGNKPQGPPPHPGKPQ		-0,02	2556,27
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PQGPPPPGGNPQQPLPPPAG		0,02	1898,98
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPP		0,00	1542,82
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGR	Gln->pyro-Glu@N-term; Phospho(S)@8	0,00	2534,12
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPH		0,03	3201,69
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PPPPGRPQGPPPPGGNPQQPLPPPAGKPQ		0,02	2853,53
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGSPSQGGNKPQGPPPHPGKPQ	Lys->Gln@24	0,03	2556,27
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGR	Gln->pyro-Glu@N-term; Deamidated(N)@4; Glu->Lys@5; Phospho(S)@8	-0,03	2534,12
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSQGPPPRPGKPE	Gln->pyro-Glu@N-term	0,01	1356,68
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPQEGNKPQRPPPPGRPQ		0,00	2130,11

99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,01	2915,34
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQ	Gln->pyro-Glu@N-term; Phospho(S)@14	0,02	2915,35
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGPPPQGGNQSQGPPP	HexNAc(N)@13	0,01	2125,01
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGGRPHRPPQGQPPQ		-0,01	1635,83
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSQGPPPHPGKPE	Gln->pyro-Glu@N-term	0,00	1337,64
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQG		0,00	2754,42
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGSPSQGGNKPQ		-0,01	1563,76
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GSPSQGGNKPQGPPPHPGKPQ		0,00	2048,02
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQ	Dehydrated(D)@6; Phospho(S)@8; Deamidated(Q)@9	0,04	2915,37
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGSPSQGGNKPQGPPPHPGKPQ	Glu->Gln@5; Deamidated(N)@13	0,02	2556,30
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQGGNQPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,10	3496,68
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGPPPHPGKPE		0,00	1139,58
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPEGPPPQ		0,01	1702,85
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSQGPPPRPGKPE		-0,12	1373,58
98,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPHPGKPE		0,01	1011,52
95,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPRPGKPE		-0,11	1245,54
95,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GKPEGRRPQGGNQPQRTPPPPGKP	Phospho(T)@17; Lys->Arg@23; Oxidation(P)@24	0,10	2656,41
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPRPGKPEGSPS		0,00	1358,70
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PPPGGNPQQPLPPPAGKPQ		0,01	1872,99
98,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPE		0,01	1226,61
96	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGPPPRPGKPE	Gln->pyro-Glu@N-term	0,01	1141,59
97,5	Basic salivary proline-rich protein 3	PRB3_HUMAN	PPPPGRPQGPPPPGGNPQ		4,00	1746,88
95,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPRPGKPE	Deamidated(Q)@2	0,01	1246,64
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GKPEGRRPQGGNQPQ		0,00	1604,81
97,5	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPEGPPPQGGNQSQG	Gly->Phe@19	0,01	2421,16
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GKPEGRRPQGGNQPQ	Lys->Phe@2	-0,08	1623,70
97,8	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPQEGNKPQRPPPPGRPQ	Oxidation(P)@2; Gln->Met@5	-0,01	2149,08
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPA		0,00	1616,83
98,5	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGPPPHPGKPE	Gln->pyro-Glu@N-term	0,01	1122,55
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPH	Deamidated(Q)@11	0,04	3202,68
95,8	Basic salivary proline-rich protein 3	PRB3_HUMAN	QPLPPPAGKPQ		0,00	1128,63

98,2	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPH	Acetyl@N-term	-0,05	1234,57
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAG		-0,01	1673,84
98,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPQEGNKPQ		0,00	1147,57
97,2	Basic salivary proline-rich protein 3	PRB3_HUMAN	GRPHRPPQGQPPQ		0,00	1450,76
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQ		-0,01	2027,05
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GGRPHRPPQGQPPQ		0,00	1507,77
97,6	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPPQGQPP		-0,02	2050,04
97,3	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPHRPPQGQPPQ		0,13	4187,30
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQ		0,00	2697,41
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPPQGQPPQ		0,00	2178,12
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQG	Deamidated(Q)@11	0,03	2755,44
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPA		-0,01	1605,78
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GKPEGRRPQGGNQPQRPPPPPGKPQ		0,01	2656,41
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQGPPPPGKPQ	Glu->Gln@13; Deamidated(Q)@18	0,01	2725,41
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQ		0,00	2016,01
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GKPQGPPQQEGNKPQ		0,00	1588,79
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQ	Deamidated(N)@8	0,01	2687,35
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQ		0,00	2686,36
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQGGNQPQ	Leu->Asn@2	-0,02	2066,02
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPA		0,01	1476,80
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQGPPPPGKPQ	Lys->Gln@25	0,05	2725,41
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPP		0,04	3150,65
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGKPQGPPPPGGNPQQPQ		-0,03	2125,04
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPAQGQ		0,00	1789,93
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPP	Pro->Ala@5	0,04	3124,63
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPAQGQQPPQ		0,00	2240,15
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPQEGNKSRSA		0,00	1323,65
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPPRPAQGQQPPQ		0,16	4238,33
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPQQEGNKPQGPPPPGKPQ		0,00	2034,02
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	LISGKPEGRRPQGGNQPQ		0,00	1918,01
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPAQ		0,00	1604,85
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPERPPPQGGNQS		0,02	2245,12
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQ		-0,13	1869,81
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQGPPPPGKPQ		0,02	2725,41
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPQGGNQSHRPPPPPGKPE		0,00	2070,04
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPPGGNPQQPQAPPAGKPQGPP	Pro->Ala@3	-0,03	2184,07
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPEGPPPQEGNKS		0,00	2218,08
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPP	Pro->Gln@4	0,06	3124,65

99	Basic salivary proline-rich protein 4	PRB4_HUMAN	QSHRPPPPPGKPE	Gln->pyro-Glu@N-term	0,01	1405,72
95	Basic salivary proline-rich protein 4	PRB4_HUMAN	SHRPPPPPGKPE		-0,08	1294,60
97	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPERPPPQGGNQSQ	Deamidated(N)@20; Gln->Asp@23	0,04	2361,15
95,5	Basic salivary proline-rich protein 4	PRB4_HUMAN	GGNQPQRPPPPPGKPQ		0,00	1650,86
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPEGPPPQEGNKS	Deamidated(Q)@2	0,03	2219,09
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPEGPPPQEGNKS	HexNAc(S)@22	0,00	2421,16
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPEGPPPQEGNKSRSA		0,01	2532,25
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPPPGKPE		0,00	1186,60
98,6	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQ		0,00	1483,83
97,4	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPQGGNQSQGPPPPPG	Deamidated(N)@8; Gln->His@9	0,05	1674,82
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQGGNQPQ	Ile->Asn@3	0,02	2066,06
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQGGNQPQ		0,00	2065,08
98,2	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPQQEGNKPQ		0,01	1178,57
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQG		0,00	2743,38
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQGGNQPQRPPPPPGKPQ		0,07	3116,74
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	APPAGKPQGPPPPPQ		-0,01	1434,75
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQ		0,00	1269,61
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQ	Pro->Ala@5	-0,01	1990,00
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFY		-0,01	1749,80
99	Histatin-1	HIS1_HUMAN	FHEKHHSHR		-0,01	1213,58
99	Histatin-1	HIS1_HUMAN	FYGDYGSNY		0,01	1084,42
98,9	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDY		0,00	2231,99
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDY		0,00	1690,73
99	Histatin-1	HIS1_HUMAN	HSHREFPFY	Deamidated(R)@4	0,00	1219,54
99	Histatin-1	HIS1_HUMAN	YGDYGSNYLYDN		0,00	1442,56
98,6	Histatin-1	HIS1_HUMAN	DSHEKRHHGYR		0,00	1420,67
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGSNYLYDN		0,04	3158,40
99	Histatin-1	HIS1_HUMAN	KHHSHREFPFYGDYGSNYLYDN		-0,03	2745,17
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSN		0,00	1948,82
99	Histatin-1	HIS1_HUMAN	SHEKRHHGY	Phospho(S)@1	0,00	1229,51
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDYGSNYLYDN		0,03	3011,33
99	Histatin-1	HIS1_HUMAN	SNYLYDN		0,02	887,39
99	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPFYGDY		-0,01	2360,07
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFY	Oxidation(H)@1; Glu->Asn@2	0,00	1750,81
99	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGS		-0,01	1697,71
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGS		0,00	1834,78

99	Histatin-1	HIS1_HUMAN	KHHSHREFPFY		-0,01	1483,70
99	Histatin-1	HIS1_HUMAN	HHSHREFPFY		0,00	1355,62
99	Histatin-1	HIS1_HUMAN	KHHSHREFPFYGDY		0,00	1818,82
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFY	Deamidated(R)@9	0,00	1897,86
95,4	Histatin-1	HIS1_HUMAN	HSHREFPFY		0,01	1218,56
97	Histatin-1	HIS1_HUMAN	DSHEKRHHGY		0,01	1264,58
95,5	Histatin-1	HIS1_HUMAN	FHEKHHSHREF	Arg->Thr@9	0,01	1434,65
95,9	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDYGSNY		0,02	2506,10
99	Histatin-1	HIS1_HUMAN	DSHEKRHHGY	Phospho(S)@2	0,00	1344,54
95,6	Histatin-1	HIS1_HUMAN	RKFHEKHHSHREFPFYGDYGSNYLYDN		0,07	3442,64
99	Histatin-1	HIS1_HUMAN	RKFHEKHHSHREFPF		0,00	2018,01
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGS		0,00	2376,05
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGSNYLYDN	Lys->Gln@4	0,05	3158,38
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPF	Deamidated(R)@8	0,03	1587,76
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFY	Deamidated(R)@8	0,02	1750,81
96,2	Histatin-1	HIS1_HUMAN	GDYGSNYLYDN		0,01	1279,51
97,2	Histatin-1	HIS1_HUMAN	EKHHSHREFPFY		-0,01	1612,75
96,9	Histatin-1	HIS1_HUMAN	HHSHREFP		0,00	1045,48
96,5	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPFYGDYGSNYLYDN		0,05	3286,51
96,7	Histatin-1	HIS1_HUMAN	FHEKHHSHR	Deamidated(R)@9	0,01	1214,58
96,3	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGSNYLYDN	Glu->Lys@3; Deamidated(N)@20	0,00	3158,40
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDYGSNYLY		0,01	2782,24
98,7	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFY		0,00	1896,88
98,4	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPF		-0,08	1861,84
99	Histatin-1	HIS1_HUMAN	EKHHSHREFPFYGDY		-0,01	1947,86
98,5	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSNY		0,00	2111,89
97,7	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSNYLY		0,02	2388,05
98,7	Histatin-1	HIS1_HUMAN	HSHREFPFYGDY		0,00	1553,67
98,9	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGSNYLYDN		0,00	2480,05
97	Histatin-1	HIS1_HUMAN	SHREFPFYGDY		-0,01	1416,60
97	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGSNY		0,00	1974,83
99	Histatin-1	HIS1_HUMAN	EKHHSHREFPF		0,01	1449,70
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFP		-0,01	1586,74
98,9	Histatin-1	HIS1_HUMAN	KFHEKHHSH		0,01	1185,58
99	Histatin-1	HIS1_HUMAN	HHSHREFPF		-0,03	1192,52
99	Histatin-1	HIS1_HUMAN	EFPFYGDYGSNYLY		0,01	1733,73
97,4	Histatin-1	HIS1_HUMAN	YGDYGSNYLY		0,00	1213,50
99	Histatin-1	HIS1_HUMAN	HREFPFYGDYGS		0,03	1473,66

99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPF		0,01	1733,83
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPF	Deamidated(R)@9	0,01	1734,81
99	Histatin-1	HIS1_HUMAN	EFPFYGDYGSNYLYDN		0,00	1962,79
99	Histatin-1	HIS1_HUMAN	HREFPFYGDYGSN		0,00	1587,67
96,6	Histatin-1	HIS1_HUMAN	SHREFPFYGDYGSNYLYDN		0,01	2342,99
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFP		0,02	1439,70
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSNYLYDN		0,00	2617,11
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPF		0,00	1586,75
99	Histatin-1	HIS1_HUMAN	EKHHSHREFPFYGDYGSNYLYDN		-0,01	2874,24
99	Histatin-1	HIS1_HUMAN	HREFPFYGDYGSNYLYDN		-0,01	2255,95
98,8	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDY		0,00	2084,92
99	Histatin-3	HIS3_HUMAN	RKFHEKHHSHRGY		0,00	1717,87
99	Histatin-3	HIS3_HUMAN	HHGYKRKF		0,02	1071,59
99	Histatin-3	HIS3_HUMAN	KFHEKHHSHRGY	Deamidated(R)@10	0,01	1562,76
99	Histatin-3	HIS3_HUMAN	SHAKRHHGY		0,01	1091,55
99	Histatin-3	HIS3_HUMAN	KRHHGYKRKFHEKHHSHRGY		0,01	2624,37
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRGYR		0,00	1589,77
99	Histatin-3	HIS3_HUMAN	SHAKRHHGYK		0,01	1219,64
99	Histatin-3	HIS3_HUMAN	HHGYKRKFHEKHHSHRGY		0,01	2340,17
99	Histatin-3	HIS3_HUMAN	YKRKFHEK		-0,09	1134,54
99	Histatin-3	HIS3_HUMAN	KRHHGYKRKFHEKHHSHR		0,00	2404,28
98,4	Histatin-3	HIS3_HUMAN	FHEKHHSHRGYR	Deamidated(R)@9; Deamidated(R)@12	0,01	1591,75
97,3	Histatin-3	HIS3_HUMAN	HEKHHSHRG		0,01	1123,55
95,4	Histatin-3	HIS3_HUMAN	KFHEKHHSHRG		-0,01	1398,70
98,2	Histatin-3	HIS3_HUMAN	HHSHRGY		0,03	892,44
96,3	Histatin-3	HIS3_HUMAN	YKRKFHEKHHS		0,00	1495,77
96,3	Histatin-3	HIS3_HUMAN	EKHHSHRGYR		0,00	1305,65
95,2	Histatin-3	HIS3_HUMAN	KRHHGYKRKF		0,00	1355,77
97,5	Histatin-3	HIS3_HUMAN	DSHAKRHHGYK		0,00	1334,66
95,6	Histatin-3	HIS3_HUMAN	HEKHHSHRGY	His->Arg@1	-0,09	1305,55
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGY		0,00	1206,56
97,3	Histatin-3	HIS3_HUMAN	GYRSNYLYDN		0,02	1263,57
97,5	Histatin-3	HIS3_HUMAN	HHGYKRKFHEK		0,00	1465,77
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRGY		0,00	1433,67
98,1	Histatin-3	HIS3_HUMAN	HEKHHSHRGYR		0,00	1442,71
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKR		0,00	1490,76
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRK	Deamidated(R)@12	0,00	1619,84

99	Histatin-3	HIS3_HUMAN	KFHEKHHSHRGY	Lys->Glu@1	0,04	1562,76
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRGY	Deamidated(R)@9	0,01	1434,66
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRK		-0,01	1618,85
99	Histatin-3	HIS3_HUMAN	KFHEKHHSHRGY		0,00	1561,77
99	Histatin-3	HIS3_HUMAN	HEKHHSHRGY		0,01	1286,61
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKFHEKHHSHRGY		0,08	3034,59
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKF		0,00	1765,92
99	Histatin-3	HIS3_HUMAN	RKFHEKHHSHRGY	Deamidated(R)@1	0,00	1718,85
99	Histatin-3	HIS3_HUMAN	HEKHHSHRGYR	Deamidated(R)@11	0,01	1443,70
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRG		0,00	1270,61
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKFHEKH		0,03	2297,21
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLLAH		0,00	1616,81
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLLAHQKPF		0,01	2117,09
99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLL		0,00	1720,89
99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLLAHQKPF	Lys->Gln@3	0,05	2429,28
99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLLA		-0,01	1791,92
98,3	Mucin-7	MUC7_HUMAN	SHFELPHYPGL		0,00	1295,63
99	Mucin-7	MUC7_HUMAN	ELPHYPGLLA		0,01	1108,60
98,8	Mucin-7	MUC7_HUMAN	MKNLLNRIIDDMVEQ		0,00	1830,94
99	Mucin-7	MUC7_HUMAN	ELPHYPGLLAHQKPF		0,00	1745,92
99	Mucin-7	MUC7_HUMAN	FELPHYPGLLAHQKPF		0,00	1892,99
99	Mucin-7	MUC7_HUMAN	SHFELPHYPG		0,00	1182,55
99	Mucin-7	MUC7_HUMAN	FELPHYPGLLA		-0,01	1255,65
99	Mucin-7	MUC7_HUMAN	FELPHYPGLL		0,00	1184,62
99	Mucin-7	MUC7_HUMAN	HHHQSPKSHFELPHYPGLL		0,02	2260,15
99	Mucin-7	MUC7_HUMAN	HFELPHYPGLL		0,00	1321,68
99	Mucin-7	MUC7_HUMAN	HHHQSPKSHFELPHYPGLLAHQKPF		0,03	2968,53
99	Mucin-7	MUC7_HUMAN	YMKNLLNRIIDD		0,00	1506,79
99	Mucin-7	MUC7_HUMAN	KSHFELPHYPGLL		0,00	1536,81
99	Mucin-7	MUC7_HUMAN	LLYMKNLLNRIIDD		0,00	1732,96
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLL		0,00	1408,71
99	Mucin-7	MUC7_HUMAN	HFELPHYPGLLA		0,00	1392,72
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLLA		0,00	1479,75
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	ASVDSGSSEEQGGSSRALVSTLVPLG		0,00	2489,22
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	AAPDEKVLDSGFREIENK		0,00	2017,01
96,5	Polymeric immunoglobulin receptor	PIGR_HUMAN	VDSGSSEEQGGSSRALVSTLVPLG		0,02	2331,18
98,6	Polymeric immunoglobulin receptor	PIGR_HUMAN	AIQDPRLFAEEK		0,00	1415,74
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	ASVDSGSSEEQGGSSRALVSTLVPL		0,01	2432,21

99	Polymeric immunoglobulin receptor	PIGR_HUMAN	SVDSGSSEEQGGSSRALVSTLVPLG		0,00	2418,19
99	Proline-rich protein 4	PROL4_HUMAN	QLSLPRFPSVSLQEAS	Gln->pyro-Glu@N-term	-0,01	1740,90
99	Proline-rich protein 4	PROL4_HUMAN	PPPEGLLPRPPGDSGNQDDGPQ		0,01	2239,06
99	Proline-rich protein 4	PROL4_HUMAN	QLSLPRFPSVS	Gln->pyro-Glu@N-term	0,00	1212,65
99	Proline-rich protein 4	PROL4_HUMAN	QLSLPRFPSVSLQ		0,00	1470,82
98,8	Proline-rich protein 4	PROL4_HUMAN	QLSLPRFPSVSLQEA	Gln->pyro-Glu@N-term	0,00	1653,87
98,7	Proline-rich protein 4	PROL4_HUMAN	QRDRPARHPQEQPLW	Gln->Arg@1; Oxidation(W)@15	0,00	1957,02
96,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGGHPPPPQGRPQ	Gln->pyro-Glu@N-term	-0,09	1462,61
97,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	1112,56
98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Ser->Ala@5	-0,09	2741,13
97	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DEERQGPPLGGQQSQPS		0,00	1808,83
97,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQGGRPQGPPQGQSPQ		0,00	1711,84
98,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPPPGKPQ		0,01	1323,70
97,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQGPPQGQSPQ		5,02	1870,93
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQ		0,04	2775,33
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHPPPPQGRPQ		0,01	1223,63
97,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@7	0,00	2604,12
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@2	0,01	2774,32
98,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQGPPQQGGHPPPPQGRPQ	Deamidated(Q)@22	0,08	3977,02
97,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RGRPQGPPQQGGHQQ		0,00	1626,80
98,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQ		0,04	2996,55
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEER		0,00	1495,61
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHQQGPPPPPPGKPQ		-0,01	1574,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQGPPQQ		0,04	3282,58
98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Dehydrated(D)@1; Phospho(S)@5; Deamidated(Q)@7	0,00	2420,98
97,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGRPQGPPQGQSPQ		4,06	1393,73
98,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQSQPSAGDGNQDDGPQ	Asp->Asn@21	0,07	2975,39
97,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	FIDEERQGPPLGGQQ		0,00	1669,81
98,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@11	0,01	2604,12
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQ		0,01	1623,68
98,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGGHPPPPQGRPQ		0,01	1479,74
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Acetyl@N-term	-0,03	2957,46
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Dehydrated(D)@4; Phospho(S)@5	-0,01	2419,98
98,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHPRPP		0,00	1604,82
98,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@12	-0,06	2604,06

98,6	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQGPPQQGGHPPPP	Deamidated(Q)@27; Gln->Asn@32	0,11	3908,93
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@7	0,07	3715,77
98,3	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHPRPP		0,01	1448,73
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQ	Deamidated(R)@2	0,01	1471,70
98,3	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHQQ	Pro->His@2	-0,02	1453,67
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGHPPPPQGRPQ		0,01	1351,68
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Dehydrated(S)@5	-0,58	2339,45
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQ		0,03	989,53
98,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQGPPQGQSP		0,00	1737,85
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Oxidation(D)@4; Glu->Asn@6	0,00	2359,03
95,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPLGGQQSQPS		0,00	1279,61
95,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQGPPQGQSPQ	Acetyl@N-term	-0,06	1907,87
95,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHP		0,00	1098,52
96	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGHQQGPPPPPPGKPQ		0,00	1702,86
98,6	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@3	0,03	3316,55
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQGPPQGQSPQ	Oxidation(P)@12; Pro->Cys@13	-0,03	1887,83
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@8	0,05	3316,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Deamidated(Q)@16; Amidated@C-term	0,06	3317,56
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQ	Asp->Asn@2	-0,01	1790,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@3	0,03	2333,03
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Phospho(S)@5; Deamidated(Q)@7	-0,01	2438,98
98,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPQGKPQ		0,00	1257,65
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Dehydrated(D)@4; Phospho(S)@5	0,05	2819,21
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@3	0,00	2204,94
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQ		0,02	1279,61
98,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQ		-0,01	2039,06
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@8	0,00	2333,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQ		0,01	1949,82
98,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	1240,62
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Deamidated(Q)@26; Amidated@C-term	0,09	3930,03
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGPQQGPPQQGGQQQQGPPPPQGKPQ		0,02	2660,29

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQ	Phospho(S)@4	0,00	2194,92
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQ	Asp->Asn@2	0,00	1662,73
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,02	2915,50
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQ	Phospho(S)@5	0,02	2309,96
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQ		0,00	1663,72
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQ		-0,02	2077,85
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQSQPS		-0,01	1921,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPPR		-0,13	1379,59
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DSEQFIDEERQGPPLGGQQ	Oxidation(D)@1; Phospho(S)@2	0,09	2225,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQ		-0,01	2205,92
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GHQQGPPPPPPGKPQ		-0,15	1517,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@7	0,00	2204,94
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLG		0,02	1929,89
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@7	0,03	2333,03
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDGNQDDGPQQGPPQQGGQQQQ		-0,02	2262,93
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQ		0,00	2333,99
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@7	0,05	3316,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EERQGPPLGGQQSQPSAGDGNQDDGPQ		0,03	2748,23
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDSEQFIDEERQGPPLGGQQ	Arg-add@N-term	-0,17	2341,92
95,3	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGRPQGPPQGQSPQ		0,00	1517,73
96,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQ		-0,02	1032,45
97,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQF	Phospho(S)@8	0,01	1232,47
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ		-0,99	3316,52
95,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	KPQGPPPQGGRPQGPPQGQSPQ		-0,01	2219,11
96,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQ	Deamidated(Q)@2; Asn->Leu@9	-0,02	2605,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@1	0,02	2774,33
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLG	Phospho(S)@4	0,01	2009,85
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDSEQFIDEERQGPPLGGQQSQPS	Arg-add@N-term	-0,15	2741,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQ	Phospho(S)@5	0,00	1703,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQ	Lys->Gln@25	0,07	2775,33
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQ	Acetyl@N-term	-0,05	1031,46
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPL	Phospho(S)@5	0,00	2067,84
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Phospho(S)@5	0,00	2438,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ		0,00	2358,04
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQSQPS	Dehydrated(S)@4	-0,55	2623,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DEERQGPPLGGQQSQPSAGDGNQDDGPQ		0,03	2863,26

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Phospho(S)@5	0,01	2837,19
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQG		0,00	1600,84
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS		0,01	2757,22
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQ	Acetyl@N-term	-0,06	2081,02
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	ERQGPPLGGQQSQPSAGDGNQDDGPQ		0,02	2619,17
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Lys->Gln@9	0,05	2915,49
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEER	Phospho(S)@4	0,00	1460,54
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSP		0,02	2787,44
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQ	Phospho(S)@4	0,00	1588,60
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Asp->Val@1	-0,16	2341,92
95,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHPRPPRGRPQ		0,00	1310,71
96,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPPPGKPQ	Gln->pyro-Glu@N-term	0,00	1306,67
95,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DEERQGPPLGGQQ		0,00	1409,65
96,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHQQ		0,00	1257,59
96,6	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SDGGDSEQFIDEERQGPPLGGQQSQPS	Asp->Gln@2	-0,16	2857,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPL	Phospho(S)@4	-0,02	1952,80
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQ		0,00	1543,81
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,05	3422,75
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQ	Dehydrated(S)@4	0,01	2225,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQ	Acetyl@N-term	-0,05	1772,82
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGRPQGPPQQGGHP		0,01	1439,71
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQG		0,00	1349,65
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Asp->Val@1	-0,11	2741,14
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQ		0,02	2243,02
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGRPQGPPQQGGHPRPP		0,00	1789,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Deamidated(Q)@7	0,02	2758,21
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQ	Phospho(S)@4	0,00	2322,97
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQ		0,04	3053,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Deamidated(Q)@25; Amidated@C-term	0,07	3716,75
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQQGGHP		0,01	2586,27
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPP		0,00	1164,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQSQPS		0,01	2642,19
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQ		0,00	1292,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRP		-0,01	1602,80
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQQ		0,01	2238,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EQFIDEERQGPPLGGQQ		0,01	1926,92
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQSQPS	Phospho(S)@4	0,01	2722,16

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	FIDEERQGPPLGGQQSQPS		0,00	2068,98
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EQFIDEERQGPPLGGQQSQPS		0,02	2326,10
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQ		0,01	2110,06
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEER		0,00	1380,58
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQ		-0,15	1730,71
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQ	Acetyl@N-term	-0,09	2562,19
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQ	Deamidated(Q)@4	0,01	1731,86
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQQGGHPRPP		0,03	2936,49
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQ		0,01	2520,29
95,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GHPPPPQGRPQ		0,01	1166,61
96,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPQGKPQGPPQQGGHPPPPQGRPQ	Gln->pyro-Glu@N-term	0,03	2953,50
98,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EERQGPPLGGQQ		0,01	1294,63
96,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQ	Gln->Lys@30	-0,01	3491,80
96,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQ	Deamidated(Q)@4	0,01	1471,70
98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ		0,01	3404,55
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPPRGRPQ		-0,16	1817,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQFIDEER	Phospho(S)@8	0,02	1874,77
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Deamidated(Q)@21	0,12	3931,04
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQ		0,00	2082,04
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RGRPQGPPQQGGHQQ	Deamidated(R)@1	0,00	1627,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQ		0,07	3491,83
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGQQQQGPPPPQGKPQ		-0,05	2135,00
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPP	Gly->Ala@1	-0,02	1237,61
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQ		0,00	2558,28
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SDGGDSEQFIDEERQGPPLGGQQ	Phospho(S)@6	0,03	2525,07
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,16	4368,34
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Deamidated(Q)@10	0,12	3931,05
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,09	3930,03
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	Oxidation(P)@3; Dehydrated(S)@4; Deamidated(Q)@10	0,09	2732,22
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSP	Deamidated(Q)@9; Oxidation(P)@33; Dioxidation(P)@34; Phospho(S)@38	-0,74	3930,08
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQ		0,02	1470,73
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPPPPGKPQGPPPQGGRPQG	Arg->Tyr@17	-0,01	1948,97
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQ	Acetyl@N-term	-0,06	1512,66
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQ	Lys->Gln@23	0,04	2520,28

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQQGGHPPPPQGRPQ		-0,06	1673,78
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQ		0,00	1522,74
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQQGGHPPPPQGRPQ	Gln->Trp@3	-0,01	1731,85
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQSQPSAGDGNQDDGPQ		0,02	2976,33
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQ		-0,01	1394,67
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHQQGPPPPPPGKPQ		0,00	2307,15
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPRGRPQGPPQQGGHQQ		0,00	1820,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Lys->Glu@9	0,08	2916,51
95,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQ		0,01	1342,65
97,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPQGKPQGPPQQGGHPPPPQGRPQ		0,02	2714,40
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQ	Asp->Asn@7	-0,01	2076,88
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	2246,08
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QFIDEERQGPPLGGQQ		-0,01	1797,85
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	KPQGPPQQGGHPPPPQGRPQ		-0,01	2084,06
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQ	Lys->Gln@20	-0,16	2262,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQSQPSAGDGNQ		0,03	2464,15
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQ		0,01	2263,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQQGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	1368,68
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGRPQGPPQQGGHPRPP	Gln->pyro-Glu@N-term	0,01	1772,89
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQQGGHPPPPQGRPQ		0,00	1576,78
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQQGGHPPPPQGRPQ	Acetyl@N-term	0,00	1715,85
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQQGPPPPQGKPQ		0,00	1385,71
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQ		-0,17	2604,93
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QPSAGDGNQDDGPQQGPPQQGGQQQQ	Pro->Cys@2; Phospho(S)@3	0,04	2732,09
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPPPQGKPQGPPQQGGHPPPPQGRPQ		0,01	2842,45
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ		-0,18	2732,98
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPLGGQQSQPSAGDGNQDDGPQ		0,01	2334,02
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGHQQ		0,00	1160,53
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHQQ		0,00	1413,68
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGHPPPPQGRPQ	Gln->pyro-Glu@N-term	0,01	1334,66
96,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EERQGPPLGGQQSQPS		0,00	1693,80
97,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPP	Acetyl@N-term	-0,05	1265,57
97,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPPRGRPQGPPQQGGHQQ		0,00	2832,41
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@11	-0,06	2732,12
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RGRPQGPPQQGGHQQGPPPPPPGKPQ		0,01	2676,39
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ		0,09	3716,77
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHQQ	Deamidated(R)@1	0,00	1414,67
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHQQGPPPPPPGKPQ		0,03	2463,28

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@12	0,02	3715,72
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SDGGDSEQFIDEERQGPPLGGQQSQPS	Phospho(S)@6	0,08	2924,29
96,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQFIDEERQGPPLGGQQ	Phospho(S)@8	0,02	2737,20
98,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,03	2761,44
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQ	Asp->Asn@1	0,00	1662,73
96,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QFIDEERQGPPLGGQQSQPS		0,03	2197,07
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@7	0,02	2732,19
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQFIDEERQGPPLGGQQSQPS	Phospho(S)@8	0,07	3136,43
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQGPPQQGGHPPPPQGRPQ		0,07	3976,03
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SDGGDSEQFIDEERQGPPLGGQQSQPS	Ser->Val@6	-0,12	2856,16
96,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPP		0,02	1223,64
97,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQ		0,01	1791,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQ	Asp->Asn@1	0,00	1790,79
98,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEER	Phospho(S)@5	0,00	1575,58
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQ	Asp->Asn@8	0,01	2076,90
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@12	0,03	2732,21
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asn->Leu@9	0,08	3715,80
99	Statherin	STAT_HUMAN	EKFLRRIGRFGYGYGPY		0,00	2078,09
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQYQQYTF		0,05	3275,58
99	Statherin	STAT_HUMAN	FGYGYGPYQPVPEQPL		-0,02	1810,83
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQYQQY		1,04	3028,45
99	Statherin	STAT_HUMAN	FGYGYGPYQPVPEQPLYPQPY		0,01	2459,15
99	Statherin	STAT_HUMAN	GPYQPVPEQPLYPQPYQPQ		-0,01	2225,07
99	Statherin	STAT_HUMAN	GYGYGPYQPVPE		0,00	1325,59
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQYT		0,05	3348,59
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPL		-0,01	1443,70
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQP		0,00	1550,71
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPL	Oxidation(P)@11; Cation:K(E)@12	-0,03	1717,71
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPY		-0,01	2091,98
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQ	Oxidation(P)@11; Cation:K(E)@12	-0,02	2719,18
99	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,08	3563,89
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQYTF	Gln->Lys@8	0,05	3495,69
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPL		-0,01	1663,78
99	Statherin	STAT_HUMAN	IGRFGYGY		0,01	931,46
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQ		0,00	2051,96
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQYTF		0,13	3495,74
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPY		-0,01	2312,07

99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPY	Oxidation(P)@11; Cation:K(E)@12	-0,03	2365,99
99	Statherin	STAT_HUMAN	YPQPYQPQYQQY		0,00	1601,71
96,5	Statherin	STAT_HUMAN	EKFLRRIGRFGY		0,01	1540,87
95,7	Statherin	STAT_HUMAN	GPYQPVPEQPLYPQPY		0,00	1871,91
97,8	Statherin	STAT_HUMAN	GRFGYGYGPY		0,00	1135,51
95,2	Statherin	STAT_HUMAN	RFGYGYGPYQPVPEQPLYPQPYQPQ		0,03	2968,45
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQ		0,01	2665,26
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPY		0,03	2785,38
98,7	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQ	Phospho(Y)@16; Tyr->His@20	-0,04	2719,17
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQY		0,06	3247,55
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,04	3138,56
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPY		-0,01	1554,77
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQYQQY		0,07	3720,84
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQYQQY		0,00	2490,18
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQ		0,00	1907,94
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQYQQYT		0,03	2591,27
99	Statherin	STAT_HUMAN	VPEQPLYPQPYQPQ		0,00	1682,83
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQYQ		0,03	3429,68
99	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPL		0,00	2562,35
99	Statherin	STAT_HUMAN	YGPYQPVPEQPL		-0,01	1386,68
99	Statherin	STAT_HUMAN	RFGYGYGPYQPVPEQPLYPQPYQPQ	Tyr->Phe@4; Oxidation(P)@8; Deamidated(Q)@10	0,03	2969,43
99	Statherin	STAT_HUMAN	YGPYQPVPEQPLYPQPYQPQ		0,01	2388,15
99	Statherin	STAT_HUMAN	RIGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,05	3294,67
99	Statherin	STAT_HUMAN	YPQPYQPQYQQYT		0,00	1702,76
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQ	Dehydrated(E)@5	0,01	1889,94
99	Statherin	STAT_HUMAN	QPYQPQYQQY		0,00	1341,60
96,9	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPL		-0,01	2137,06
96,5	Statherin	STAT_HUMAN	GPYQPVPEQPL		0,00	1223,62
98,7	Statherin	STAT_HUMAN	IGRFGYGYGP		0,01	1085,54
99	Statherin	STAT_HUMAN	YQPVPEQPLYPQPYQPQ		0,01	2071,01
99	Statherin	STAT_HUMAN	VPEQPLYPQPYQPQYQQY		0,02	2265,09
97,1	Statherin	STAT_HUMAN	IGRFGYGYGPY		0,00	1248,59
97,4	Statherin	STAT_HUMAN	YPQPYQPQYQQYTF		-0,02	1849,81
96,7	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPL	Oxidation(P)@9; Val->His@10	-0,07	1717,70

96,7	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQ	Phospho(Y)@14; Tyr->His@18	-0,02	2499,10
97,8	Statherin	STAT_HUMAN	YQPVPEQPLYPQPYQPQYQQYT		0,03	2754,33
99	Statherin	STAT_HUMAN	PQPYQPQYQQYTF		0,01	1686,78
96,6	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQ	Deamidated(Q)@1	0,00	1908,93
97,1	Statherin	STAT_HUMAN	LRRIGRFGYGYGPY		-0,01	1673,87
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQ	Cation:Na(E)@12	-0,11	2687,12
97	Statherin	STAT_HUMAN	QPQYQQYTF		0,01	1201,55
97,8	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPLYPQPYQPQYQQY	Deamidated(Q)@33	0,14	4147,18
99	Statherin	STAT_HUMAN	GPYQPVPEQPLYPQPYQPQYQQY		0,00	2807,33
95,5	Statherin	STAT_HUMAN	YGPYQPVPE		0,01	1048,50
97,4	Statherin	STAT_HUMAN	YPQPYQPQYQQ		0,00	1438,65
99	Statherin	STAT_HUMAN	GRFGYGYGPYQPVPEQPLYPQPY		0,03	2672,29
98,2	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQP		0,00	2023,98
98,9	Statherin	STAT_HUMAN	PEQPLYPQPYQPQ		0,01	1583,77
97,9	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQYQQYTF		0,12	3969,00
98,6	Statherin	STAT_HUMAN	RFGYGYGPYQPVPEQPLYPQPYQPQYQQY		0,02	3550,68
98,5	Statherin	STAT_HUMAN	YGPYQPVPEQPLYPQPY		0,00	2034,97
98,4	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQ		-3,09	2442,07
99	Statherin	STAT_HUMAN	EQPLYPQPYQPQ		0,01	1486,72
98,7	Statherin	STAT_HUMAN	VPEQPLYPQPY		0,00	1329,66
99	Submaxillary gland androgen-regulated protein 3A	SMR3A_HUMAN	GPYPPGPLAPPPPPC	Dioxidation(C)@15	-0,06	1487,65
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGIFPPPPPQP		0,01	1199,64
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPYGPGR		-0,01	2322,19
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGPG		0,01	1525,81
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPG		0,01	1133,60
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPGRIPPPPPAPY		0,01	2614,39
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPGF		0,00	1280,66
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGIFPPPPPQ		-0,03	1102,55
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPF		0,00	1433,73
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPG		-0,01	1644,82
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAP		0,01	1151,65
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPY	Oxidation(P)@7	0,01	1330,72
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPGRIPPPPPAPYGPGIFPPPPPQP		0,07	3796,07
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGF		-0,01	1791,89
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	IPPPPPAPYGPGIFPPPPPQP		0,00	2129,14
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPY		0,01	2733,41
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	IPPPPPAPYGPGIFP		-0,01	1515,80
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,04	3518,89

99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPGF	Gln->pyro-Glu@N-term	-0,01	2369,20
97,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPF	Gln->pyro-Glu@N-term	0,00	2011,05
97	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGP		0,01	1468,79
97,1	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY	Deamidated(R)@31	0,15	4031,23
95,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	VPPPPPPPYGPGRIPPPPPAPYGPGIFPPPPPQP		0,08	3437,91
96,6	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPG		0,01	866,43
97,5	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGF		0,02	1013,51
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	VPPPPPPPYGPGRIPPPPPAPY		-0,01	2256,20
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPF		0,00	1279,66
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPGFVPPPPPPPY		0,00	2222,16
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPG		0,00	1490,76
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPRGPYPPGPLAPPQPF		0,00	1743,91
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY	Deamidated(Q)@12	0,14	4031,22
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGF		0,00	1637,82
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPG		0,05	2944,55
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGFVPPPPPPPY		0,00	2579,32
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGR		0,04	3100,63
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,13	4030,22
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PGIFPPPPPQP		0,00	1142,61
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GRIPPPPPAPYGPGIFPPPPPQP		0,03	2342,29
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPP	Gln->pyro-Glu@N-term	0,02	1106,58
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PGRIPPPPPAPYGPGIFPPPPPQP	Gly->Arg@2	-0,09	2538,30
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPL	Gln->pyro-Glu@N-term	-0,05	1373,68
97,9	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGPGIFPPPPPQP		0,00	2496,34
97,9	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PGRIPPPPPAPY		0,02	1257,71
97,9	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGPGIFP		0,01	1883,02
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPG	Gln->pyro-Glu@N-term	-0,01	2222,13
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPGFVPPPPPPPY	Gln->pyro-Glu@N-term	0,07	3310,77
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGFVPPPPPPPYGPGR		0,06	2946,58
98,5	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPY		0,02	1314,73
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPY	Pro->Ala@18; Phospho(Y)@19	-0,04	2008,91
96,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPY	Oxidation(P)@8	0,00	1330,70
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGP		0,00	1471,75
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PGFVPPPPPPPY		0,00	1260,66
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPY		-0,01	1954,99
97,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQP		0,00	1286,67
96,5	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PPPPPPPY		0,02	860,46
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPYGPG		-0,01	2166,08

96,7	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGP	Gln->pyro-Glu@N-term	0,00	1260,64
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPY		0,00	1317,68
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,04	3251,73
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,10	3876,12
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPG		0,00	1528,77
98,8	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	VPPPPPPPYGPGR		0,02	1326,72
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	FVPPPPPPPYGPGRIPPPPPAPY		0,03	2403,31
98,9	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	RGPYPPGPLAPPQPFGPGFVPPPPPPPY		0,03	2889,53
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPGR		0,00	1684,87
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	FVPPPPPPPYGPGRIPPPPPAPYGPGIFPPPPPQP		0,11	3585,01

Supplementary table S3: Salivary peptides identified in all groups, common to all DM-related groups and exclusive to T1D-R+N, T1D-R, T1D and Ctrl.

Conf	Protein Name	Accession Number	Sequence	Modifications	ΔMass	Prec MW
Salivary peptides identified in all groups						
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQGPPPPPGKPQ	Deamidated(N)@2; Pro->Asp@3	0,00	2433,16
98,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPA		-0,01	1676,82
98,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQGPPPPGKPQ	Gln->Pro@5	-0,01	1959,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQ		-0,16	1839,72
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNQPQGPPPPPGKPQ		0,00	1551,78
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQ		0,06	3148,60
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNPQGPSPQGGNKPQ		0,00	1461,70
96,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQ		0,08	3963,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPAQGGSKSQSARAPPGKPQ		0,00	2002,04
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQ		0,00	1349,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQ		0,00	1340,65
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPP		0,00	2338,15
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPP		0,04	2688,39
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	Deamidated(N)@9	0,04	2817,43
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS		0,08	3270,72
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQSPPGKPQ	Pro->Asp@4; Deamidated(Q)@5	-0,01	2059,98
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@21	-0,01	3085,57
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ		0,00	2883,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSP		0,00	1221,61
96,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQ		0,00	1075,54
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPR		0,00	1377,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSRSPQSPPGKPQGPPPQGGNQPQ		0,04	3098,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQ		-0,01	1930,99
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQ		0,00	2028,01
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQ		-0,01	2133,98
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQ	Acetyl@N-term	-0,01	1117,51
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQ	Glu->Gln@6; Deamidated(N)@9	0,02	2487,17

97,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,01	3484,66
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPQQGGNRPQ		0,07	3144,63
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,07	3233,59
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQ	Gln->Lys@12	0,05	3777,00
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPSRPPPQ		0,00	1679,90
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPQGGRPSRPPPQ		0,00	1272,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QPQAPPAGQPQGPP		-0,01	1368,67
98,1	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQP	Asn->Lys@15	0,00	1652,87
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQ		-0,16	1766,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQ	Lys->Gln@26	0,05	2719,39
98,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPQQGGNRPQ		0,00	2087,07
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SARAPPGKPQGPPQQEGNNPQ		0,01	2154,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSQSPRSPPGKPQ		-0,01	2041,03
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPPRPPQ		0,00	1493,77
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGDKSQSPRSPPGKPQGPPPQGGNQPQ		0,05	3098,58
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQ		0,01	1185,62
98,1	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQ		0,01	1134,56
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GNPQGAPPQGGNKPQGPPSPPGKPQ		0,00	2388,19
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSARSPPGKPQ	Lys->Gln@19	0,03	2129,05
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSRSS		-0,14	2180,95
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKSQGPPPPGKPQ	HexNAc(N)@8	-0,01	2124,05
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQ		-0,17	1855,71
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,16	3925,05
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,15	4176,16
98,3	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPE	Acetyl@N-term	-0,05	1268,56
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPH		0,03	3201,69
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PPPPGRPQGPPPPGGNPQQPLPPPAGKPQ		0,02	2853,53
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGR	Gln->pyro-Glu@N-term; Phospho(S)@8	0,00	2534,12
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPQEGNKPQRPPPPGRPQ		0,00	2130,11
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,01	2915,34
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGPPPHPGKPE		0,00	1139,58
98,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPHPGKPE		0,01	1011,52
95,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPRPGKPE		-0,11	1245,54
96	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGPPPRPGKPE	Gln->pyro-Glu@N-term	0,01	1141,59
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GKPEGRRPQGGNQPQ		0,00	1604,81

99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPA		0,00	1616,83
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAG		-0,01	1673,84
98,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPQEGNKPQ		0,00	1147,57
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GGRPHRPPQGQPPQ		0,00	1507,77
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQ		-0,01	2027,05
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQ		0,00	2697,41
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPPQGQPPQ		0,00	2178,12
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQGPPPPGKPQ	Glu->Gln@13; Deamidated(Q)@18	0,01	2725,41
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQ		0,00	2016,01
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQGGNQPQ	Leu->Asn@2	-0,02	2066,02
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPA		0,01	1476,80
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPP		0,04	3150,65
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPAQGQQPPQ		0,00	2240,15
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SPPGKPQGPPQQEGNKPQ		-0,13	1869,81
98,2	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPQQEGNKPQ		0,01	1178,57
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQ		0,00	1269,61
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFY		-0,01	1749,80
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDY		0,00	1690,73
99	Histatin-1	HIS1_HUMAN	YGDYGSNYLYDN		0,00	1442,56
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGS		0,00	1834,78
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPF	Deamidated(R)@8	0,03	1587,76
98,7	Histatin-1	HIS1_HUMAN	HSHREFPFYGDY		0,00	1553,67
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPF		0,01	1733,83
97,5	Histatin-3	HIS3_HUMAN	DSHAKRHHGYK		0,00	1334,66
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRGY		0,00	1433,67
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLLAHQKPF		0,01	2117,09
99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLL		0,00	1720,89
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLL		0,00	1408,71
99	Mucin-7	MUC7_HUMAN	SHFELPHYPGLLA		0,00	1479,75
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	ASVDSGSSEEQGGSSRALVSTLVPLG		0,00	2489,22
98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQSQPS	Ser->Ala@5	-0,09	2741,13
97,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQGGRPQGPPQGQSPQ		0,00	1711,84
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQ		0,04	2775,33
97,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQGPPQGQSPQ		5,02	1870,93

98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQQ	Dehydrated(D)@1; Phospho(S)@5; Deamidated(Q)@7	0,00	2420,98
98,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQ		0,04	2996,55
97,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RGRPQGPPQQGGHQQ		0,00	1626,80
97,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGRPQGPPQGQSPQ		4,06	1393,73
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQ		0,01	1623,68
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Acetyl@N-term	-0,03	2957,46
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@7	0,07	3715,77
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQ	Deamidated(R)@2	0,01	1471,70
98,3	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHPRPP		0,01	1448,73
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPQGGRPQ		0,03	989,53
98,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGHPPPPQGRPQ		0,01	1351,68
95,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPLGGQQSQPS		0,00	1279,61
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQ	Asp->Asn@3	0,03	2333,03
98,6	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ	Asp->Asn@3	0,03	3316,55
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQ	Asp->Asn@2	-0,01	1790,79
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQQQ	Asp->Asn@3	0,00	2204,94
98,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPQGKPQ		0,00	1257,65
98,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQGGRPQ		-0,01	2039,06
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQ	Phospho(S)@4	0,00	2194,92
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ	Deamidated(Q)@26; Amidated@C-term	0,09	3930,03
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQSQPS		-0,01	1921,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQSQPS	Dehydrated(S)@4	-0,55	2623,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEER	Phospho(S)@4	0,00	1460,54
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQ	Phospho(S)@4	0,00	1588,60
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPPPPPGKPQGPPPQ		0,00	1543,81
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGDSEQFIDEERQGPPLGGQQ	Dehydrated(S)@4	0,01	2225,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQ	Acetyl@N-term	-0,05	1772,82
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGRPQGPPQQGGHPRPP		0,00	1789,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQ		0,04	3053,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQ	Acetyl@N-term	-0,09	2562,19
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQQGGHPRPP		0,03	2936,49
95,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GHPPPPQGRPQ		0,01	1166,61
96,7	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQ	Gln->Lys@30	-0,01	3491,80
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPPRGRPQ		-0,16	1817,79

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPP	Gly->Ala@1	-0,02	1237,61
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQ		0,00	2082,04
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GRPQGPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,16	4368,34
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	Oxidation(P)@3; Dehydrated(S)@4; Deamidated(Q)@10	0,09	2732,22
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPQQGGHPPPPQGRPQ		-0,06	1673,78
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQQ		0,00	1522,74
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	2246,08
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQQGPPPPQGKPQ	Gln->pyro-Glu@N-term	0,00	1368,68
96,1	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EERQGPPLGGQQSQPS		0,00	1693,80
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPL		-0,01	1443,70
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPL	Oxidation(P)@11; Cation:K(E)@12	-0,03	1717,71
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQ	Oxidation(P)@11; Cation:K(E)@12	-0,02	2719,18
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQYTF	Gln->Lys@8	0,05	3495,69
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPY		-0,01	2312,07
99	Statherin	STAT_HUMAN	YPQPYQPQYQQY		0,00	1601,71
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPY		0,03	2785,38
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,04	3138,56
99	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPLYPQPYQPQYQQY		0,07	3720,84
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPY		-0,01	1554,77
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQ		0,00	1907,94
99	Statherin	STAT_HUMAN	YGPYQPVPEQPLYPQPYQPQ		0,01	2388,15
99	Statherin	STAT_HUMAN	YPQPYQPQYQQYT		0,00	1702,76
96,9	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQPL		-0,01	2137,06
96,7	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQ	Phospho(Y)@14; Tyr->His@18	-0,02	2499,10
97,1	Statherin	STAT_HUMAN	IGRFGYGYGPY		0,00	1248,59
97,4	Statherin	STAT_HUMAN	YPQPYQPQYQQYTF		-0,02	1849,81
98,2	Statherin	STAT_HUMAN	IGRFGYGYGPYQPVPEQP		0,00	2023,98
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGIFPPPPPQP		0,01	1199,64
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGPG		0,01	1525,81
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPGRIPPPPPAPY		0,01	2614,39
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPF		0,00	1433,73
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAP		0,01	1151,65
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPY	Oxidation(P)@7	0,01	1330,72

99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPG		-0,01	1644,82
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPY		0,01	2733,41
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPGF	Gln->pyro-Glu@N-term	-0,01	2369,20
97	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGP		0,01	1468,79
97,1	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY	Deamidated(R)@31	0,15	4031,23
97,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPF	Gln->pyro-Glu@N-term	0,00	2011,05
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPGFVPPPPPPPY		0,00	2222,16
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPF		0,00	1279,66
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPG		0,00	1490,76
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQPFGPGFVPPPPPPPYGPGR		0,04	3100,63
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGFVPPPPPPPY		0,00	2579,32
97,9	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGRIPPPPPAPYGPGIFPPPPPQP		0,00	2496,34
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPG	Gln->pyro-Glu@N-term	-0,01	2222,13
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGPLAPPQPFGPGFVPPPPPPPY	Gln->pyro-Glu@N-term	0,07	3310,77
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPY	Pro->Ala@18; Phospho(Y)@19	-0,04	2008,91
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,04	3251,73
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPY		0,00	1317,68
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	FVPPPPPPPPYGPGRIPPPPPAPY		0,03	2403,31
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGPG		0,00	1528,77
Common T1D-R+N, T1D-R and T1D						
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSQSPRSPPGKPQ		0,06	2896,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPA		0,01	2823,34
95,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPS		-0,03	1201,60
96,6	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSRSPR		-0,06	2233,11
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GQPQGPPRPPQGGRPS		0,00	1611,83
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPP		0,08	3834,94
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PSLIAGNPQGP		0,03	1049,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QNLNEDVSQEESPSLIAGNPQGPSPQGGNKPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,08	3378,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	RPPQGGRPSRPPQ		0,00	1428,77
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQ		-0,14	1978,85
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSQSARSPPGKPQGPPPQGGNQPQ		0,08	3186,63
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPPPGKPE		0,00	1186,60
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGSNYLYDN		0,04	3158,40
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDYGSNYLYDN		0,03	3011,33

97	Histatin-1	HIS1_HUMAN	DSHEKRHHGY		0,01	1264,58
96,2	Histatin-1	HIS1_HUMAN	GDYGSNYLYDN		0,01	1279,51
96,9	Histatin-1	HIS1_HUMAN	HHSHREFP		0,00	1045,48
96,5	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPFYGDYGSNYLYDN		0,05	3286,51
98,9	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGSNYLYDN		0,00	2480,05
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFP		-0,01	1586,74
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFP		0,02	1439,70
99	Histatin-1	HIS1_HUMAN	EKHHSHREFPFYGDYGSNYLYDN		-0,01	2874,24
99	Histatin-3	HIS3_HUMAN	KFHEKHHSHRGY	Deamidated(R)@10	0,01	1562,76
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKR		0,00	1490,76
99	Mucin-7	MUC7_HUMAN	ELPHYPGLLAHQKPF		0,00	1745,92
99	Salivary acidic proline-rich phosphoprotein ½	PRPC_HUMAN	DGPQQGPPQQGGQQQQGPPPPQGKPQ		0,02	2660,29
95,2	Statherin	STAT_HUMAN	RFGYGYGPYQPVPEQPLYPQPYQPQ		0,03	2968,45
99	Statherin	STAT_HUMAN	RIGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,05	3294,67
Exclusive to T1D-R+N						
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	AGNPQGPSPQGGNKPQ	Ser->Pro@8	0,00	1542,75
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPPRPPQGGRPS		0,00	1948,00
96,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPP		0,00	1318,71
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKSQS	Ser->Met@29; Deamidated(Q)@30; Phospho(S)@31	0,15	3134,62
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQ		0,03	2504,29
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ	Deamidated(N)@29; Amidated@C-term	0,09	3941,10
97,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	GNKPQGPPPPGKPQ		-0,02	1397,72
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PSPQGGNKPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ	Phospho(S)@2; Gln->Asp@4	0,13	3941,07
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGGNKPQGPPPPPGKPQ	Gln->pyro-Glu@N-term	0,00	1662,85
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGPPPQGGNKPQGPPPPGKPQ	Gln->Cys@1	0,02	2034,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SLIAGNPQGPSPQGGNKPQ		0,00	1845,93
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQG	Lys->Ala@37	0,16	3777,03
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQ		0,08	3162,60
98,5	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSR		0,02	1151,58
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	AGNPQGAPPQGGNKPQ	Ala->Pro@7	0,00	1542,75
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQSA		-0,05	2137,00
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDNK	Lys->Thr@29	0,03	2794,41

99	Basic salivary proline-rich protein 2	PRB2_HUMAN	QNLNEDVSQEESPSLIAGNPQGAPPQGGNKPQ	Gln->pyro-Glu@N-term; Phospho(S)@8; Ala->Ser@23	0,07	3378,57
96,8	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKSQS	Gln->Tyr@20; Phospho(S)@21	-0,03	2180,96
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	QGGNKPQGPPSPPGKPQGPPPQGGNQPQ	Gln->pyro-Glu@N-term	0,08	2710,40
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	SRSPPGKPQGPPPQGGNQPQ	Arg->Asp@2	0,00	1968,93
96,1	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGDNKS		-0,12	1850,80
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GGNKSQGPPPPGKPQ	HexNAc(N)@3	0,00	1647,82
95,6	Basic salivary proline-rich protein 3	PRB3_HUMAN	SGKPEGRRPQGGNQPQ		0,00	1691,84
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGPPPQGGNQSQGPPP	HexNAc(N)@13	0,01	2125,01
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QGGRPHRPPQGQPPQ		-0,01	1635,83
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	QSLNEDVSQEESPSVISGKPEGRRPQGGNQPQ	Gln->pyro-Glu@N-term; Phospho(S)@8	0,10	3496,68
97,5	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPEGPPPQGGNQSQG	Gly->Phe@19	0,01	2421,16
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GKPQGPPQQEGNKPQ		0,00	1588,79
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPPRPAQGQQPPQ		0,16	4238,33
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPPQGGRPPRPAQ		0,00	1604,85
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPQGGNQSHRPPPPPGKPE		0,00	2070,04
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	QSHRPPPPPGKPE	Gln->pyro-Glu@N-term	0,01	1405,72
97	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPERPPPQGGNQSQ	Deamidated(N)@20; Gln->Asp@23	0,04	2361,15
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPEGPPPQEGNKSRSA		0,01	2532,25
98,6	Basic salivary proline-rich protein 4	PRB4_HUMAN	FLISGKPEGRRPQ		0,00	1483,83
95,5	Histatin-1	HIS1_HUMAN	FHEKHHSHREF	Arg->Thr@9	0,01	1434,65
99	Histatin-1	HIS1_HUMAN	RKFHEKHHSHREFPF		0,00	2018,01
98,4	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPF		-0,08	1861,84
99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLLA		-0,01	1791,92
99	Mucin-7	MUC7_HUMAN	ELPHYPGLLA		0,01	1108,60
99	Mucin-7	MUC7_HUMAN	KSHFELPHYPGLL		0,00	1536,81
99	Mucin-7	MUC7_HUMAN	HFELPHYPGLLA		0,00	1392,72
99	Proline-rich protein 4	PROL4_HUMAN	PPPEGLLPRPPGDSGNQDDGPQ		0,01	2239,06
95,3	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGRPQGPPQGQSPQ		0,00	1517,73
97,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQF	Phospho(S)@8	0,01	1232,47
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPL	Phospho(S)@5	0,00	2067,84
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQ		0,01	2110,06
96,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QQGPPPPQGKPQGPPQQGGHPPPPQGRPQ	Gln->pyro-Glu@N-term	0,03	2953,50

99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSP	Deamidated(Q)@9; Oxidation(P)@33; Dioxidation(P)@34; Phospho(S)@38	-0,74	3930,08
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPPPPGKPQGPPPQGGRPQG	Arg->Tyr@17	-0,01	1948,97
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	IDEERQGPPLGGQ		-0,01	1394,67
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQYQQYTF		0,05	3275,58
99	Statherin	STAT_HUMAN	GYGPYQPVPEQPLYPQPYQPQYQQY		1,04	3028,45
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQ		0,00	2051,96
97,8	Statherin	STAT_HUMAN	GRFGYGYGPY		0,00	1135,51
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	IPPPPPAPYGPGIFP		-0,01	1515,80
95,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	VPPPPPPPYGPGRIPPPPPAPYGPGIFPPPPPQP		0,08	3437,91
Exclusive to T1D-R						
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNKPQGPPPPPGKPQGPPPQGGNKPQ		-0,01	2609,34
98,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNQPQGPPPPPGKPQGPPQQ		0,01	2059,03
96,2	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSQSPR	Lys->Arg@17	0,03	2233,16
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GGNKPQGPPPPPGKPQ		-0,04	1551,77
97,8	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPQQ		0,00	1477,76
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQ		0,01	1323,71
98	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPAQGGSKSQ	Lys->Gln@9	0,05	2925,50
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQ		0,02	970,54
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPPGKPQGPPPQ		0,01	1446,77
98,5	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQ		0,05	3195,67
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PQGPPPPGKPQGPPPQ	Gln->Pro@2	0,00	1543,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	QGPPPPPGKPQGPPPQ	Gln->Pro@1	0,00	1543,81
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ		0,11	3909,01
98,3	Basic salivary proline-rich protein 2	PRB2_HUMAN	QGGNKPQGPPSPPGKPQ	Asn->Pro@4; Deamidated(Q)@17	0,02	1653,86
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	SQGPPPHPGKPEGPPPQ		0,01	1702,85
97,4	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPQGGNQSQGPPPPPG	Deamidated(N)@8; Gln->His@9	0,05	1674,82
99	Histatin-1	HIS1_HUMAN	KHHSHREFPFYGDYGSNYLYDN		-0,03	2745,17
99	Histatin-1	HIS1_HUMAN	SHEKRHHGY	Phospho(S)@1	0,00	1229,51
99	Histatin-1	HIS1_HUMAN	SNYLYDN		0,02	887,39
99	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDYGS		0,00	2376,05
99	Histatin-1	HIS1_HUMAN	HEKHHSHREFPFYGDYGSNYLY		0,01	2782,24
97,7	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSNYLY		0,02	2388,05

97	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGSNY		0,00	1974,83
99	Histatin-1	HIS1_HUMAN	EFPFYGDYGSNYLY		0,01	1733,73
97,4	Histatin-1	HIS1_HUMAN	YGDYGSNYLY		0,00	1213,50
99	Histatin-1	HIS1_HUMAN	EFPFYGDYGSNYLYDN		0,00	1962,79
96,6	Histatin-1	HIS1_HUMAN	SHREFPFYGDYGSNYLYDN		0,01	2342,99
99	Histatin-3	HIS3_HUMAN	HHGYKRKF		0,02	1071,59
99	Histatin-3	HIS3_HUMAN	SHAKRHHGY		0,01	1091,55
99	Histatin-3	HIS3_HUMAN	SHAKRHHGYK		0,01	1219,64
99	Histatin-3	HIS3_HUMAN	YKRKFHEK		-0,09	1134,54
98,2	Histatin-3	HIS3_HUMAN	HHSHRGY		0,03	892,44
97,3	Histatin-3	HIS3_HUMAN	GYRSNYLYDN		0,02	1263,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHPPPPQGRPQ		0,01	1223,63
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHQQGPPPPPPGKPQ		-0,01	1574,79
98,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	RPQGPPQQGGHPRPP		0,00	1604,82
95,5	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DEERQGPPLGGQQ		0,00	1409,65
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GGHQQGPPPPPPGKPQGPPPQGGRPQGPPQGQSPQ		0,05	3422,75
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQG		0,00	1349,65
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGRPQGPPQQGGHP		0,01	1439,71
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QPSAGDGNQDDGPQQGPPQQGGQQQQ	Pro->Cys@2; Phospho(S)@3	0,04	2732,09
99	Statherin	STAT_HUMAN	PQPYQPQYQQYTF		0,01	1686,78
95,5	Statherin	STAT_HUMAN	YGPYQPVPE		0,01	1048,50
99	Submaxillary gland androgen-regulated protein 3A	SMR3A_HUMAN	GPYPPGPLAPPPPPC	Dioxidation(C)@15	-0,06	1487,65
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PGIFPPPPPQP		0,00	1142,61
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPP	Gln->pyro-Glu@N-term	0,02	1106,58
96,5	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	PPPPPPPY		0,02	860,46
96,7	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	QRGPRGPYPPGP	Gln->pyro-Glu@N-term	0,00	1260,64
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	YPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,10	3876,12

Exclusive to T1D

Conf	Protein Name	Accession Number	Sequence	Modifications	ΔMass	Prec MW
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPAGQPQGPP		0,01	1015,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	ESPSLIAGNPQGPSPQGGNKPQ		-0,01	2159,05
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	ESPSLIAGNPQGPSPQGGNKPQGPPPPPGKPQ	Deamidated(N)@9	0,02	3112,58
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQGGSKSQ		-0,39	1867,56
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQG		0,04	2873,47
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQ		0,10	4004,10

96,4	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPRSPPGKPQGPPPQGGNQPQ		0,01	2107,06
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPPGKPQGPPPQGDKSRSPQSPPGKPQ		0,02	2896,52
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDKSRS	Deamidated(Q)@25; Oxidation(R)@30; Phospho(S)@31	0,15	3134,64
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	SPPGKPQGPPPQGGNQPQGPPPPPG		0,00	2366,18
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQGGNRPQGPPPPGKPQGPPPQGDKS	Gln->Pro@5	0,05	2822,47
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPRPPQGGRPSRPP		0,00	1551,84
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	KPQGPPPQGGNKPQ		0,00	1428,75
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGDNKSRSSRSPPGKPQ		0,00	2173,09
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGSKSRSS		0,00	1240,62
97,1	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPPGKPQGPPPQGGSKSR		-0,01	1922,01
97,4	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGSKSRSA		0,01	1224,63
98	Basic salivary proline-rich protein 2	PRB2_HUMAN	SPPGKPQGPPQQE		0,01	1345,67
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PQGPPPPGGNPQQPLPPPAG		0,02	1898,98
95,9	Basic salivary proline-rich protein 3	PRB3_HUMAN	GKPEGRRPQGGNQPQRTPPPPGKP	Phospho(T)@17; Lys->Arg@23; Oxidation(P)@24	0,10	2656,41
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPRPGKPEGSPS		0,00	1358,70
95,8	Basic salivary proline-rich protein 3	PRB3_HUMAN	QPLPPPAGKPQ		0,00	1128,63
97,2	Basic salivary proline-rich protein 3	PRB3_HUMAN	GRPHRPPQGQPPQ		0,00	1450,76
97,3	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPHRPPQGQPPQ		0,13	4187,30
97,6	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPPQGQPP		-0,02	2050,04
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	GPPPPGGNPQQPQAPPAGKPQGPPPPPQG		0,00	2743,38
98,9	Histatin-1	HIS1_HUMAN	FHEKHHSHREFPFYGDY		0,00	2231,99
99	Histatin-1	HIS1_HUMAN	KFHEKHHSHREFPFYGDY		-0,01	2360,07
95,6	Histatin-1	HIS1_HUMAN	RKFHEKHHSHREFPFYGDYGSNYLYDN		0,07	3442,64
99	Histatin-3	HIS3_HUMAN	KRHHGYKRKFHEKHHSHRGY		0,01	2624,37
99	Histatin-3	HIS3_HUMAN	HHGYKRKFHEKHHSHRGY		0,01	2340,17
99	Histatin-3	HIS3_HUMAN	KRHHGYKRKFHEKHHSHR		0,00	2404,28
96,3	Histatin-3	HIS3_HUMAN	EKHHSHRGYR		0,00	1305,65
95,2	Histatin-3	HIS3_HUMAN	KRHHGYKRKF		0,00	1355,77
97,5	Histatin-3	HIS3_HUMAN	HHGYKRKFHEK		0,00	1465,77
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKF		0,00	1765,92
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKFHEKHHSHRGY		0,08	3034,59
99	Histatin-3	HIS3_HUMAN	DSHAKRHHGYKRKFHEKH		0,03	2297,21
99	Histatin-3	HIS3_HUMAN	FHEKHHSHRG		0,00	1270,61

99	Mucin-7	MUC7_HUMAN	SPKSHFELPHYPGLLAHQKPF	Lys->Gln@3	0,05	2429,28
98,8	Mucin-7	MUC7_HUMAN	MKNLLNRIIDDMVEQ		0,00	1830,94
99	Mucin-7	MUC7_HUMAN	FELPHYPGLLAHQKPF		0,00	1892,99
99	Mucin-7	MUC7_HUMAN	HHHQSPKSHFELPHYPGLL		0,02	2260,15
99	Mucin-7	MUC7_HUMAN	HHHQSPKSHFELPHYPGLLAHQKPF		0,03	2968,53
99	Mucin-7	MUC7_HUMAN	LLYMKNLLNRIIDD		0,00	1732,96
98,6	Polymeric immunoglobulin receptor	PIGR_HUMAN	AIQDPRLFAEEK		0,00	1415,74
96,5	Polymeric immunoglobulin receptor	PIGR_HUMAN	VDSGSSEEQGGSSRALVSTLVPLG		0,02	2331,18
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	ASVDSGSSEEQGGSSRALVSTLVPL		0,01	2432,21
99	Polymeric immunoglobulin receptor	PIGR_HUMAN	SVDSGSSEEQGGSSRALVSTLVPLG		0,00	2418,19
98,7	Proline-rich protein 4	PROL4_HUMAN	QRDRPARHPQEQPLW	Gln->Arg@1; Oxidation(W)@15	0,00	1957,02
95,2	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQGPPQQGGHP		0,00	1098,52
96	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGGHQQGPPPPPPGKPQ		0,00	1702,86
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DGGDSEQFIDEERQGPPLGGQ	Phospho(S)@5	0,02	2309,96
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDGNQDDGPQQGPPQQGGQQQQ		-0,02	2262,93
95,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	KPQGPPPQGGRPQGPPQGQSPQ		-0,01	2219,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPP		0,00	1164,57
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPPPPQGRPQGPPQQ		0,01	2238,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	VISDGGDSEQFIDEER	Phospho(S)@8	0,02	1874,77
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPLGGQQSQPSAGDGNQDDGPQ		0,01	2334,02
97,4	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GPPQQGGHPRPPRGRPQGPPQQGGHQQ		0,00	2832,41
99	Statherin	STAT_HUMAN	GYGYGPYQPVPEQPLYPQPYQPQYQQYT		0,05	3348,59
99	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPLYPQPYQPQ		0,08	3563,89
97,8	Statherin	STAT_HUMAN	YQPVPEQPLYPQPYQPQYQQYT		0,03	2754,33
97,8	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPLYPQPYQPQYQQY	Deamidated(Q)@33	0,14	4147,18
98,6	Statherin	STAT_HUMAN	RFGYGYGPYQPVPEQPLYPQPYQPQYQQY		0,02	3550,68
99	Statherin	STAT_HUMAN	EQPLYPQPYQPQ		0,01	1486,72
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPY		0,04	3518,89
96,6	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPG		0,01	866,43
99	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPGFVPPPPPPPYGP		0,00	1471,75
Exclusive to Controls						
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	APPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPP		0,19	4160,21
98,3	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPGKPQGPPPQGGNQPQ		-0,15	1679,69
99	Basic salivary proline-rich protein 1	PRP1_HUMAN	PPPAGGNPQQPQAPPAGQPQGPP		-0,01	2184,06
98,7	Basic salivary proline-rich protein 1	PRP1_HUMAN	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS		0,14	4417,29
99	Basic salivary proline-rich protein 2	PRB2_HUMAN	GPPPQGGNKPQGPPPPGKPQGPPPQGDNKS		0,01	2908,47

99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPHRPP		0,00	1542,82
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PGKPEGSPSQGGNKPQ		-0,01	1563,76
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	GSPSQGGNKPQGPPPHPGKPQ		0,00	2048,02
99	Basic salivary proline-rich protein 3	PRB3_HUMAN	PPPGGNPQQPLPPPAGKPQ		0,01	1872,99
98,2	Basic salivary proline-rich protein 3	PRB3_HUMAN	GPPPPPQGGRPH	Acetyl@N-term	-0,05	1234,57
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	SQGPPPHPGKPERPPPQGGNQS		0,02	2245,12
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPPGGNPQQPQAPPAGKPQGPP	Pro->Ala@3	-0,03	2184,07
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	PPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPP	Pro->Gln@4	0,06	3124,65
95,5	Basic salivary proline-rich protein 4	PRB4_HUMAN	GGNQPQRPPPPPGKPQ		0,00	1650,86
99	Basic salivary proline-rich protein 4	PRB4_HUMAN	APPAGKPQGPPPPPQ		-0,01	1434,75
99	Histatin-1	HIS1_HUMAN	HHSHREFPFYGDYGSN		0,00	1948,82
99	Histatin-1	HIS1_HUMAN	HSHREFPFYGDYGS		-0,01	1697,71
99	Histatin-1	HIS1_HUMAN	KHHSHREFPFY		-0,01	1483,70
99	Histatin-1	HIS1_HUMAN	KHHSHREFPFYGDY		0,00	1818,82
99	Histatin-1	HIS1_HUMAN	EKHHSHREFPFYGDY		-0,01	1947,86
99	Histatin-1	HIS1_HUMAN	HREFPFYGDYGS		0,03	1473,66
99	Histatin-1	HIS1_HUMAN	HREFPFYGDYGSN		0,00	1587,67
99	Mucin-7	MUC7_HUMAN	SHFELPHYPG		0,00	1182,55
98,6	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DDGPQQGPPQQGGQQQQGPPPPQGKPQGPPQQGGHPPPP	Deamidated(Q)@27; Gln->Asn@32	0,11	3908,93
98,9	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGQQQQ		0,02	1279,61
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	AGDGNQDDGPQQGPPQQGGQ		0,01	1949,82
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DSEQFIDEERQGPPLGGQQ	Oxidation(D)@1; Phospho(S)@2	0,09	2225,01
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDSEQFIDEERQGPPLGGQQ	Arg-add@N-term	-0,17	2341,92
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	GDSEQFIDEERQGPPLGGQQSQPS	Arg-add@N-term	-0,15	2741,11
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	DEERQGPPLGGQQSQPSAGDGNQDDGPQ		0,03	2863,26
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EQFIDEERQGPPLGGQQ		0,01	1926,92
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	EQFIDEERQGPPLGGQQSQPS		0,02	2326,10
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	FIDEERQGPPLGGQQSQPS		0,00	2068,98
98	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	SAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQ		0,01	3404,55
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PPRGRPQGPPQQGGHQQ		0,00	1820,91
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	PQQGGHPPPPQGRPQ		0,00	1576,78
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPPPQGKPQGPPQQGGHPPPPQGRPQ		0,01	2842,45
99	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QGPPQQGGHQQ		0,00	1160,53
96,8	Salivary acidic proline-rich phosphoprotein 1/2	PRPC_HUMAN	QFIDEERQGPPLGGQQSQPS		0,03	2197,07

99	Statherin	STAT_HUMAN	FGYGYGPYQPVPEQPLYPQPY	0,01	2459,15
99	Statherin	STAT_HUMAN	IGRFGYGY	0,01	931,46
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQYQQY	0,00	2490,18
99	Statherin	STAT_HUMAN	QPVPEQPLYPQPYQPQYQQYT	0,03	2591,27
99	Statherin	STAT_HUMAN	LRRIGRFGYGYGPYQPVPEQPL	0,00	2562,35
99	Statherin	STAT_HUMAN	GPYQPVPEQPLYPQPYQPQYQQY	0,00	2807,33
98,5	Statherin	STAT_HUMAN	YGPYQPVPEQPLYPQPY	0,00	2034,97
97,5	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	APPQPFGPGF	0,02	1013,51
97,3	Submaxillary gland androgen-regulated protein 3B	SMR3B_HUMAN	GPYPPGPLAPPQP	0,00	1286,67

Supplementary table S4: Number of amino acid residues in P1 and P1′ positions for the identified salivary peptides *per* group and protein class.

T1D-R+N								T1D-R								T1D								Ctrl							
N-terminal				C-terminal				N-terminal				C-terminal				N-terminal				C-terminal				N-terminal				C-terminal			
P1		P1'		P1		P1'		P1		P1'		P1		P1'		P1		P1'		P1		P1'		P1		P1'		P1		P1'	
Basic salivary proline-rich protein 1 (bPRP1)																															
Q	37	G	36	Q	29	G	35	Q	12	G	11	Q	9	G	9	Q	6	G	7	Q	7	G	7	Q	9	G	9	S	9	P	11
P	9	P	9	P	11	Q	9	R	1	S	2	R	2	A	1	S	3	P	2	E	2	E	2	P	3	R	5	P	2	G	2
G	7	Q	9	G	9	S	8					K	1	P	1	G	2	R	2	R	2	S	2	S	2			Q	2	A	1
S	4	R	2	R	6	A	4					P	1	S	1	P	2	K	1	G	1	A	1					R	1		
		A	1	I	1	P	1							Q	1			Q	1	Q	1	K	1								
				E	1																										
Basic salivary proline-rich protein 2 (bPRP2)																															
Q	6	G	6	Q	8	G	8	Q	1	G	1	P	1	Q	1	R	2	G	2	Q	4	G	4	S	1	R	1	Q	1	G	1
S	3	S	3	S	2	Q	2									A	1	R	2	R	1	S	1								
K	2	R	2	A	1	S	2									E	1	S	2												
A	1	A	1	I	1	A	1									Q	1														
R	1	Q	1	P	1											S	1														
Basic salivary proline-rich protein 3 (bPRP3)																															
								Q	2	E	1	Q	1	S	1	P	2	Q	2	Q	4	G	5	Q	3	G	3	Q	2	G	3
										G	1					G	1	E	1	G	1	P	1	H	1	Q	1	E	1	P	2
																Q	1	G	1	R	1	Q	1	P	1	R	1	P	1			
																S	1	K	1	S	1							R	1			
Basic salivary proline-rich protein 4 (bPRP4)																															
Q	4	G	4	Q	4	G	3	G	1	K	1	Q	1	G	1	G	1	G	1	Q	1	G	1	P	2	G	2	Q	4	P	2
E	2	R	3	L	1	S	2																	Q	2	H	1	G	2	S	2
A	1			N	1	F	1																	S	2	P	1			A	1
				P	1	P	1																			Q	1			G	1
				R	1	Q	1																			R	1				
Histatin 1																															
F	3	Y	2	R	2	F	1	Y	6	D	4	R	3	S	4	Y	2	G	2	R	2	F	1	Y	3	G	3	E	2	H	4
		P	1	K	1	K	1	S	1	L	1	F	2	H	3					K	1	K	1	N	2	N	2	H	2	K	2
						R	1			N	1	H	2	E	2							R	1	S	2	Y	2	S	2	E	1
										R	1	K	2	F	1													K	1		
												D	1	K	1																
												E	1	Y	1																
												G	1																		
Histatin 3																															
								K	2	H	2	D	2	S	2	Y	3	H	4	A	6	D	3								
								Y	2	R	2	R	2	H	2	F	2	R	3	R	2	K	3								
								F	1	K	1	G	1	G	1	R	2	Y	1	H	1	H	2								
												K	1	Y	1	G	1	S	1	K	1	E	1								
																H	1	G	1			F	1								

																K	1														
Mucin-7 (MUC7)																															
A	3	H	3	F	1	E	1									F	3	I	3	R	2	H	2	G	1	L	1	K	1	S	1
L	1	A	1	P	1	H	1									D	1	A	1	F	1	F	1								
				Q	1	K	1									L	1	M	1	H	1	L	1								
				S	1	S	1													Y	1	M	1								
Polymeric immunoglobulin receptor (PIgR)																															
																G	2	L	2	A	1	A	2								
																K	1	A	1	K	1	S	1								
																L	1	G	1	R	1	V	1								
																				S	1										
Proline-rich protein 4 (PROL4)																															
Q	1	Q	1	R	1	P	1																								
Salivary acidic proline-rich phosphoprotein 1/2 (aPRP)																															
Q	4	G	3	Q	4	G	4									Q	6	G	4	Q	4	G	4	Q	11	G	6	Q	4	Q	4
P	2	R	3	G	1	Q	2									P	3	Q	4	R	2	P	2	S	4	A	4	P	3	D	3
G	1	S	1	I	1	D	1									R	1	P	1	A	1	Q	2	P	1	S	3	G	3	P	2
				P	1	R	1											R	1	G	1	D	1			Q	3	S	3	G	2
				S	1															K	1	K	1					I	1	E	2
																				L	1	V	1					E	1	S	1
																				S	1							R	1	F	1
																														A	1
Statherin																															
Y	2	P	1	Y	2	G	4	E	1	Q	1	G	1	P	1	Q	2	F	2	F	3	L	2	Y	5	Q	2	Y	3	Q	2
Q	1	Q	1	F	1							Y	1	Y	1	T	2	T	2	P	2	E	1	L	1	T	2	R	2	F	1
		T	1	I	1											Y	2	Y	2	G	1	G	1	T	1	F	1	F	1	G	1
																						R	1			G	1	G	1	I	1
																						Y	1			Y	1			L	1
																														Y	1
Submaxillary gland androgen-regulated protein 3B (SMR3B)																															
P	1	P	1	F	1	I	1	P	2	G	3	S	2	P	2	G	1	G	2	F	1	G	2	F	1	F	1	L	1	A	1
				R	1	V	1	Y	2	L	1	G	1	Q	2	P	1	F	1	L	1	A	1	P	1	V	1	R	1	G	1
												P	1	Y	1	Y	1			P	1										

Total																													
Q	50	G	46	Q	42	G	50	Q	15	G	16	Q	11	G	11	Q	16	G	21	Q	22	G	24	Q	25	G	24	S	14	P	17
P	10	P	12	P	14	S	13	Y	8	D	4	R	7	S	8	G	8	R	8	R	13	K	6	S	11	R	8	Q	13	G	11
G	7	Q	12	R	11	Q	12	K	2	R	3	G	4	H	5	P	8	Q	7	A	8	S	5	P	8	Q	7	G	6	Q	6
S	7	R	7	G	9	A	5	P	2	H	2	K	4	P	4	Y	8	H	4	F	5	A	4	Y	8	A	4	P	6	A	4
A	5	A	3	F	3	P	3	E	1	K	2	D	3	Q	4	F	5	F	3	G	4	D	4	N	2	S	3	R	6	H	4
F	3	H	3	I	3	F	2	F	1	L	2	P	3	Y	4	R	5	I	3	K	4	E	4	F	1	Y	3	E	4	S	4
E	2	S	3	S	3	K	2	G	1	S	2	F	2	E	2	S	5	P	3	P	3	H	4	G	1	F	2	Y	3	D	3
K	2	Y	2	Y	2	E	1	R	1	N	1	H	2	A	1	K	2	S	3	S	3	F	3	H	1	N	2	H	2	E	3
Y	2	T	1	A	1	H	1	S	1	Q	1	S	2	F	1	L	2	Y	3	E	2	L	3	L	1	T	2	K	2	F	2
L	1			E	1	I	1			E	1	E	1	K	1	T	2	A	2	H	2	P	3	T	1	H	1	F	1	K	2
R	1			K	1	R	1					V	1			A	1	K	2	L	2	Q	3			L	1	I	1	I	1
				L	1	V	1					Y	1			D	1	L	2	Y	1	R	2			P	1	L	1	L	1
				N	1											E	1	T	2			V	2			V	1			Y	1
																H	1	E	1			M	1								
																		M	1			Y	1								

P1 precedes P1’ on the parent protein sequence, i.e. proteolytic cleavage occurs between P1 and P1’.

Supplementary table S5: Distribution of the identified peptides presenting Gln->pyro-Glu at N-term and phosphorylation *per* group. Data is presented as mean ± STD.

	% **Gln->pyro-Glu at N-term**				**% Phosphorylation**				**% HexNAc**			
Peptides	**T1D-R**+N	**T1D-R**	**T1D**	**Ctrl**	**T1D-R**+N	**T1D-R**	**T1D**	**Ctrl**	**T1D-R**+N	**T1D-R**	**T1D**	**Ctrl**
Basic salivary proline-rich protein 1 (bPRP1)	1.18±1.67	0.85±1.20	0.37±0.52	-	1.69±0.94	0.88±1.24	1.37±0.89	-	-	-	-	-
Basic salivary proline-rich protein 2 (bPRP2)	0.87±1.23	-	0.42±0.59	-	1.41±0.53	0.61±0.86	1.19±1.68	-	1.81±1.09	1.93±0.53	0.97±1.37	0.76±1.07
Basic salivary proline-rich protein 3 (bPRP3)	17.29±3.83	9.95±14.07	8.69±2.86	10.07±1.99	7.50±4.71	3.13±4.42	7.50±1.18	1.47±2.08	1.56±2.21	-	-	-
Basic salivary proline-rich protein 4 (bPRP4)	4.85±3.91	2.88±4.07	1.67±2.36	2.27±3.21	-	-	-	-	0.71±1.01	-	-	-
Histatin 1	-	-	-	-	-	3.57±5.05	-	-	-	-	-	-
Histatin 3	-	-	-	-	-	-	-	-	-	-	-	-
Mucin-7 (MUC7)	-	-	-	-	-	-	-	-	-	-	-	-
Polymeric immunoglobulin receptor (PIgR)	-	-	-	-	-	-	-	-	-	-	-	-
Proline-rich protein 4 (PROL4)	37.50±17.68	12.50±17.68	-	16.67±23.57	-	-	-	-	-	-	-	-
Salivary acidic proline-rich phosphoprotein 1/2 (aPRP)	5.74±0.69	0.36±0.51	1.12±1.58	3.03±0.49	15.83±2.50	15.10±0.73	17.00±5.42	11.99±1.06	-	-	-	-
Statherin	-	-	-	-	-	1.89±2.67	-	1.61±2.28	-	-	-	-
Submaxillary gland androgen-regulated protein 3B (SMR3B)	7.44±4.37	11.86±2.08	7.41±10.48	7.02±1.68	-	0.83±1.18	-	-	-	-	-	-

(-)- The modification was not detected.

Supplementary table S6: Salivary peptides differentially regulated between T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl (Conf refers to confidence level of Protscore from ProteinPilot; ∆Mass in Da).

Name	Conf	Sequence	∆Mass	Theor m/z	T1D-R+N /Ctrl	T1D-R /Ctrl	T1D /Ctrl
Basic salivary proline-rich protein 1 (bPRP1)							
	98	GPPPQGGNKPQGPPPPGKPQ	0.02	2844.62	5.92±0.03	6.58±0.02	6.64±0.06
	99	GPPQQEGNNPQGPPPPAGGNPQQPQ	0.05	2792.37	4.54±0.02	1.17±0.00	2.18±0.02
	98	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ[#]GPP	0.03	3790.85	4.19±0.02	1.76±0.00	2.66±0.02
	99	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ	0.04	3538.74	4.07±0.02	1.70±0.00	3.19±0.03
	99	GPPQQEGNNPQGPPPPAGGN	-0.01	2214.08	3.95±0.02	1.65±0.00	2.69±0.02
	99	GPPPQGGNQPQGPPPPPGKPQ	0.00	2637.44	3.53±0.02	2.52±0.01	3.02±0.03
	97	GPPQQE[¢]GNN[#]PQGPPPPAGGNPQQPQAPPAGQPQGPP	0.17	3789.86	3.34±0.02	1.71±0.00	1.89±0.02
	99	GPPQQEGNNPQGPPPPAGGNPQ	0.00	2439.20	3.32±0.02	0.84±0.00	1.14±0.01
	99	APPGKPQGPPQQEGNNPQ	0.07	2449.30	2.59±0.01	2.27±0.01	3.81±0.03
	99	GPPPPAGGNPQQPQ	-0.01	1645.86	2.54±0.01	0.61±0.00	1.64±0.01
	99	GGNQPQGPPPPPGKPQ	0.00	2161.20	2.50±0.01	1.94±0.01	1.23±0.01
	96	GPPPP[Ł]AGGNPQQPQAPPAGQPQGPPRPPQ	-0.27	3123.64	2.44±0.01	1.47±0.00	3.45±0.03
	99	GPPPPAGGNPQQPQAPPA	-0.02	1982.04	2.37±0.01	1.00±0.00	1.72±0.02
	99	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	-0.02	3121.62	2.02±0.01	1.52±0.00	3.04±0.03
	95	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS	-0.05	3575.85	1.98±0.01	0.79±0.00	1.07±0.01
	98	GPPPPPGK[§]PQ	0.04	1579.94	1.69±0.01	0.79±0.00	1.41±0.01
	99	GPPQQEGNNPQ	0.00	1469.73	1.58±0.01	0.60±0.00	1.32±0.01
	98	GPPPPPGKPQ	0.00	1275.74	1.56±0.01	0.89±0.00	1.22±0.01
	95	GNPQGPSPQGGNKPQ	-0.04	1792.92	1.51±0.01	0.58±0.04	1.75±0.22
	99	GPPPQGGNK[§]PQ	0.00	1684.96	1.44±0.01	0.90±0.00	1.48±0.01
	99	GPPQQEGNN[♯]PQ	-0.29	1787.99	1.38±0.01	0.49±0.00	0.50±0.00
	99	GPPPPAGGNPQQPQAPPAGQPQ	0.01	2392.23	1.37±0.01	0.81±0.00	1.51±0.01
	99	GPPPPAGGNPQQPQAPPAGQPQGPP	-0.04	2643.36	1.31±0.01	0.56±0.00	1.63±0.01
	95	GPPPPAGGNPQ	0.00	1292.69	1.29±0.01	0.68±0.00	1.35±0.01
	96	[I]APPGKPQGPPQQEGNNPQ	-0.03	2187.11	1.22±0.01	1.51±0.00	1.96±0.02

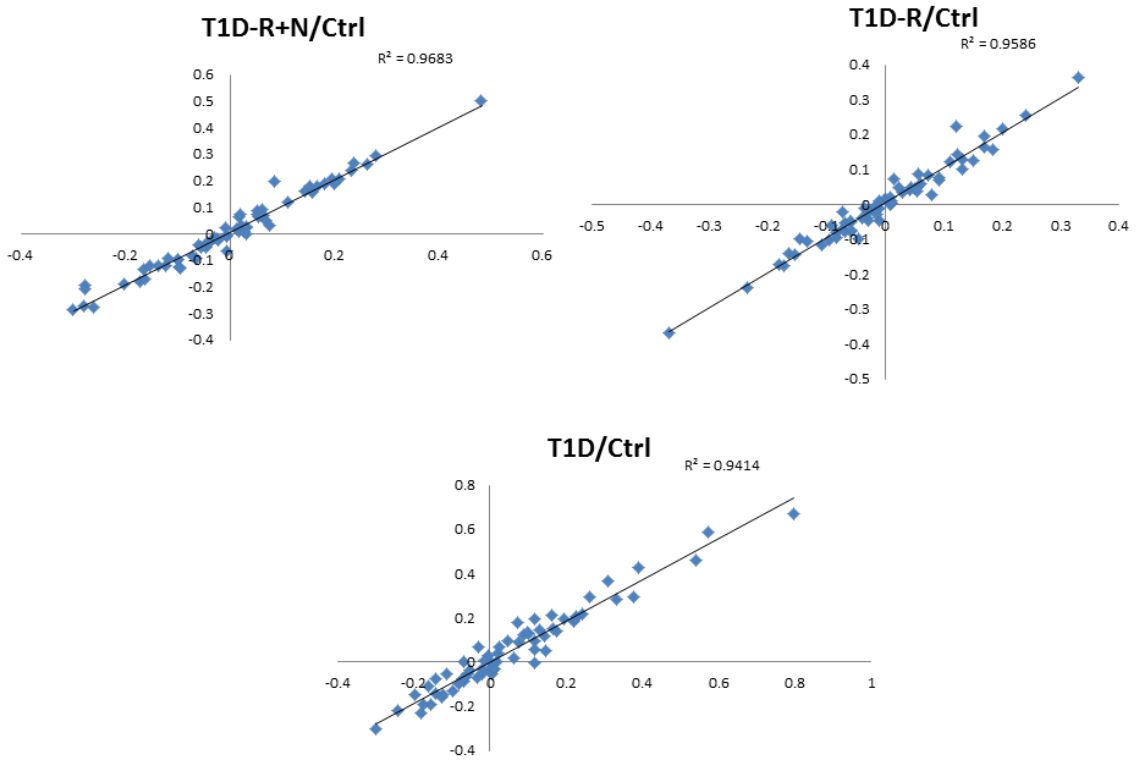
	96	GNPQGPSPQGGNK[§]PQ	-0.01	2071.11	1.06±0.01	0.53±0.00	1.23±0.01
	98	GPPPQGGNKPQ	0.00	1423.76	1.01±0.01	0.57±0.00	1.74±0.02
	99	[ŀ]GNPQGPSPQGGNK[§]PQ	-0.01	1809.91	0.80±0.00	0.53±0.00	1.60±0.01
	98	GK[§]PQGPPPQGGNQPQ	-0.02	2095.15	0.79±0.00	0.46±0.00	0.59±0.01
Basic salivary proline-rich protein 2 (bPRP2)							
	98	GPPPQGGNK[§]PQGPPPPGK[§]PQ	0.02	2844.62	5.92±0.03	6.58±0.02	6.64±0.06
	99	GPPQQEGNNPQGPPPPAGGNPQQPQ	0.05	2792.37	4.54±0.02	1.17±0.00	2.18±0.02
	98	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ[#]GPP	0.03	3790.85	4.19±0.02	1.76±0.00	2.66±0.02
	99	GPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ	0.04	3538.74	4.07±0.02	1.70±0.00	3.19±0.03
	99	GPPQQEGNNPQGPPPPAGGN	-0.01	2214.08	3.95±0.02	1.65±0.00	2.69±0.02
	99	GPPPQGGNQPQGPPPPPGK[§]PQ	0.00	2637.44	3.53±0.02	2.52±0.01	3.02±0.03
	97	GPPQQE[¢]GNN[#]PQGPPPPAGGNPQQPQAPPAGQPQGPP	0.17	3789.86	3.34±0.02	1.71±0.00	1.89±0.02
	99	GPPQQEGNNPQGPPPPAGGNPQ	0.00	2439.20	3.32±0.02	0.84±0.00	1.14±0.01
	99	GPPPPAGGNPQQPQ	-0.01	1645.86	2.54±0.01	0.61±0.00	1.64±0.01
	99	GGNQPQGPPPPPGKPQ	0.00	2161.20	2.50±0.01	1.94±0.01	1.23±0.01
	96	GPPPP[Ł]AGGNPQQPQAPPAGQPQGPPRPPQ	-0.27	3123.64	2.44±0.01	1.47±0.00	3.45±0.03
	99	GPPPPAGGNPQQPQAPPA	-0.02	1982.04	2.37±0.01	1.00±0.00	1.72±0.02
	99	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQ	-0.02	3121.62	2.02±0.01	1.52±0.00	3.04±0.03
	95	GPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPS	-0.05	3575.85	1.98±0.01	0.79±0.00	1.07±0.01
	98	GPPPPPGK[§]PQ	0.04	1579.94	1.69±0.01	0.79±0.00	1.41±0.01
	98	GPPPPPGKPQ	0.00	1275.74	1.56±0.01	0.89±0.00	1.22±0.01
	99	SPPGK[§]PQGPPPQGGNQPQ	0.00	2376.29	1.49±0.01	1.36±0.00	2.91±0.03
	99	GPPPQGGNKPQ	0.00	1684.96	1.44±0.01	0.90±0.00	1.48±0.01
	99	SPPGK[§]PQGPPPQGGNQPQ	-0.05	2098.10	1.43±0.01	1.47±0.00	2.88±0.03
	99	GPPQQEGN[#]NPQ	-0.29	1787.99	1.38±0.01	0.49±0.00	0.50±0.00
	99	[ŀ]SPPGK[§]PQGPPPQGGNQPQ	0.01	2115.09	1.37±0.01	1.04±0.00	4.26±0.04
	99	GPPPPAGGNPQQPQAPPAGQPQ	0.01	2392.23	1.37±0.01	0.81±0.00	1.51±0.01
	99	GPPPPAGGNPQQPQAPPAGQPQGPP	-0.04	2643.36	1.31±0.01	0.56±0.00	1.63±0.01
	95	GPPPPAGGNPQ	0.00	1292.69	1.29±0.01	0.68±0.00	1.35±0.01
	99	GPPQQEGNNPQ	0.00	1469.73	1.23±0.01	0.46±0.00	1.09±0.01
	99	SPPGKPQGPPPQGGNQPQ	0.00	2072.08	1.15±0.01	0.93±0.00	0.71±0.01

	99	GNPQGAPPQGGNK[§]PQ	-0.02	2055.12	1.01±0.01	0.55±0.00	1.50±0.01
	98	[t]GPPPQGGNKPQ	0.00	1423.76	1.01±0.01	0.57±0.00	1.74±0.02
	98	GK[§]PQGPPPQGGNQPQ	-0.02	2095.15	0.79±0.00	0.46±0.00	0.59±0.01
	98	[t]GNPQGAPPQGGNK[§]PQ	-0.01	1793.92	0.66±0.00	0.48±0.00	2.25±0.02
Basic salivary proline-rich protein 3 (bPRP3)							
	98	SQGPPPH[ŧ]PGK[§]PE	0.00	1796.02	2.26±0.11	0.88±0.08	0.82±0.21
	98	SQGPPPR[¥]PGK[§]PE	0.00	1796.02	2.26±0.11	0.88±0.08	0.82±0.21
	98	[t]GPPPPPQGGRPHRPPQGQPPQ	0.08	2222.13	2.03±0.01	2.51±0.01	2.94±0.03
	99	GPPPPPQGGRPHRPPQGQPPQ	0.07	2483.33	1.68±0.01	6.50±0.02	6.77±0.06
	99	GPPP[ɳ]QEGNK[§]PQ	0.00	1787.99	1.47±0.01	0.57±0.00	0.48±0.00
	95	[¤]QGPPPHPGK[§]PE	0.00	1427.76	1.45±0.01	0.64±0.00	0.64±0.01
	96	GRPHRPPQGQPPQ	0.00	1755.97	1.41±0.01	1.37±0.00	5.25±0.05
	97	GGRPHRPPQGQPPQ	-0.01	1812.99	1.12±0.01	0.91±0.00	1.67±0.01
	97	[t]GPPPPGGNPQQPLPPPAGK[§]PQ	-0.01	2375.28	1.10±0.01	0.86±0.00	1.84±0.02
	99	GPPPPGGNPQQPLPPPAGK[§]PQ	0.00	2636.48	1.03±0.01	0.91±0.00	1.28±0.01
	95	[t]GPPPPPQGGRPHRPPQGQPPQ	-0.06	2222.13	0.92±0.01	0.79±0.00	0.99±0.01
	98	[¤]QSQGPPPRPGK[§]PE	0.00	1661.89	0.87±0.00	0.45±0.00	0.39±0.00
	96	SQGPPPRPGKPE	-0.02	1855.06	0.78±0.00	0.33±0.00	0.63±0.01
	97	GPPPQEGNKPQRPPPPGRPQ	-0.92	2739.53	0.73±0.00	0.94±0.00	2.13±0.02
Histatin 1							
	99	GDYGSNYLYDN	-0.01	1584.71	0.81±0.02	1.32±0.02	1.60±0.01
	99	GDYGSNYLYDN[#]	-0.10	1585.70	0.82±0.00	1.23±0.00	1.59±0.01
	99	YGDYGSNYLYDN	-0.01	1747.78	0.86±0.00	1.18±0.00	1.85±0.02
Salivary acidic proline-rich phosphoprotein 1/2 (aPRP)							
	96	GPPPQGGRPQGPPQGQSPQ	-0.02	2171.13	2.10±0.01	11.17±0.03	12.61±0.11
	99	SQPSAGDGNQDDGPQQGPPQQ	0.01	2412.10	2.08±0.01	1.58±0.00	1.38±0.01
	99	SQPSAGDGNQDDGPQQGPPQQGGQ	-0.02	2654.20	1.99±0.01	0.60±0.00	0.52±0.00
	99	SQPSAGDGNQDDGPQQGPPQQGGQQ	0.03	2782.26	1.95±0.01	0.50±0.00	0.55±0.00
	99	AGDGNQDDGPQQGPPQQGGQ	0.01	2255.02	1.65±0.01	0.41±0.00	0.88±0.01
	98	QGPPPPPPGK[§]PQ	0.01	1483.82	1.65±0.01	0.93±0.00	1.15±0.01
	99	AGDGNQDDGPQQGPPQQGGQQQ	-0.03	2511.14	1.56±0.01	0.78±0.00	0.66±0.01

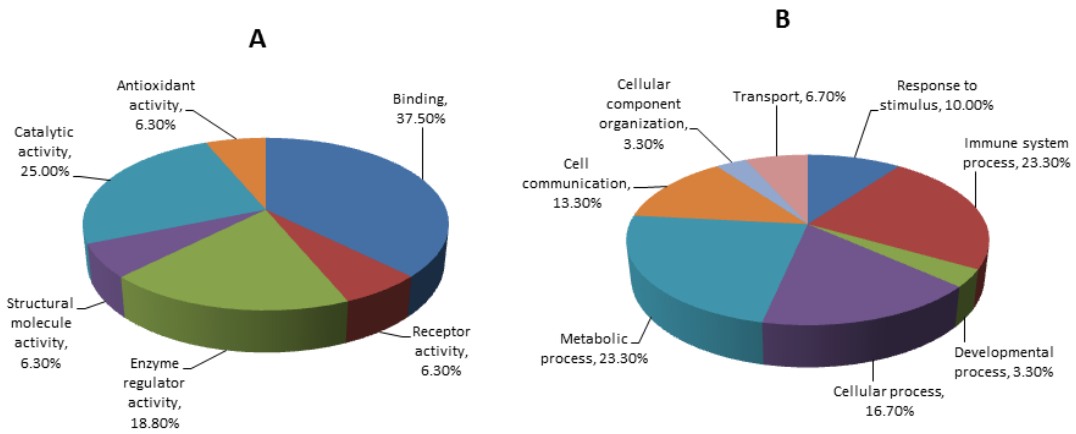
	99	QQQGPPPPQGK[§]PQ	-0.01	1673.89	1.51±0.01	0.56±0.00	0.61±0.01
	99	SQPSAGDGNQDDGPQQGPPQQGGQQQ	0.02	2910.32	1.38±0.01	0.47±0.00	0.35±0.00
	99	SQPSAGDGNQDDGPQQGPPQQGGQQ[#]Q	0.05	2911.30	1.29±0.01	0.53±0.00	0.30±0.00
	99	AGD[ω]GNQDDGPQQGPPQQGGQ	-0.04	2254.04	1.14±0.01	0.46±0.00	0.84±0.01
	99	SQPSAGDGNQDD[ω]GPQQGPPQQGGQQQ	0.02	2909.33	1.14±0.01	0.53±0.00	0.31±0.00
	99	QGPPQQGGQQQQGPPPPQGK[§]PQ	-0.01	2551.30	1.09±0.01	0.48±0.00	0.62±0.01
	98	QGPPPPQGK[§]PQ	0.00	1417.77	1.09±0.01	0.56±0.00	0.39±0.00
	99	PQGPPQQGGHPPPPQGRPQ	0.06	2261.18	1.07±0.01	1.05±0.00	1.59±0.01
	99	GPPPPPPGKPQ	0.00	1372.79	1.06±0.01	0.80±0.00	1.24±0.01
	99	QQGPPPPQGK[§]PQ	0.00	1545.83	1.06±0.01	0.49±0.00	0.60±0.01
	99	SAGDGNQDDGPQQGPPQQGGQQQ	0.04	2598.17	1.06±0.01	0.27±0.00	0.32±0.00
	95	QGPPPPQGKPQ	0.05	1739.01	1.05±0.01	1.09±0.00	1.14±0.01
	99	GPPPQGGRPQGPPQGQSP	-0.02	2043.07	0.85±0.00	2.11±0.01	4.60±0.04
	95	GPPPQGGRPQ	0.00	1294.72	0.84±0.00	0.61±0.00	1.33±0.01
	99	AGDGNQDDGPQQGPPQQGGQQ	-0.01	2383.08	0.83±0.00	0.39±0.00	0.45±0.00
	99	GPPQQGGHPRPP	-0.07	1528.83	0.82±0.01	0.49±0.04	1.06±0.01
	99	SAGDGNQD[ω]DGPQQGPPQQGGQQQ	-0.02	2597.19	0.82±0.00	0.30±0.00	0.18±0.00
	99	SQPSAGDGNQD[ω]DGPQQGPPQQGGQQQQ	-0.01	3037.39	0.81±0.00	0.51±0.00	0.70±0.01
	99	SQPSAGDGNQDDGPQQGPPQQGGQQQQ	0.01	3038.37	0.80±0.00	0.41±0.00	0.85±0.01
	99	GPPQQGGHQQ	0.03	1337.69	0.80±0.00	1.10±0.00	0.85±0.01
	99	SAGDGNQDDGPQQGPPQQGGQQQQ	0.01	2726.23	0.73±0.00	0.27±0.00	0.45±0.00
	99	GPPQQGGHPPPPQGRPQ	0.00	2036.07	0.72±0.00	0.44±0.00	0.80±0.01
	97	GPPPPQGK[§]PQ	0.04	1610.95	0.67±0.00	0.53±0.00	0.86±0.01
	99	QQGPPPPQGKPQ	-0.02	1867.06	0.67±0.00	0.79±0.00	0.78±0.01
	99	DDGPQQGPPQQGGQQQ	-0.01	1968.93	0.60±0.00	0.74±0.00	1.06±0.01
	99	AGDGNQDDGPQQGPPQQGGQQQQ	-0.03	2639.20	0.58±0.00	0.36±0.00	0.82±0.01
	99	SAGDGNQDD[ω]GPQQGPPQQGGQQQQ	0.05	2725.25	0.52±0.00	0.26±0.00	0.34±0.00
	99	DGPQQGPPQQGGQQQQ	-0.02	1981.96	0.50±0.00	0.32±0.00	0.90±0.01
	99	DDGPQQGPPQQGGQQQQ	-0.02	2096.99	0.49±0.00	0.56±0.00	0.91±0.01
	99	AGDGN[ɣ]QDDGPQQGPPQQGGQQQQ	-0.04	2638.24	0.32±0.00	0.46±0.00	0.48±0.00
	99	DD[ω]GPQQGPPQQGGQQQQ	-0.01	2096.01	0.27±0.00	0.45±0.00	0.61±0.01
Statherin							
	96	GYGYGPYQPVPEQPLYPQPY	0.09	2617.29	1.31±0.01	1.48±0.00	1.31±0.01
	99	GYGPYQPVPEQPLYPQPYQPQ	0.08	2750.38	1.15±0.01	1.00±0.00	1.70±0.02

	99	YGYGPYQPVPEQPLYPQP	0.09	2397.21	1.12±0.01	1.47±0.00	1.12±0.01
	99	GYGYGPYQPVPEQPL	-0.02	1969.00	1.05±0.01	0.98±0.00	1.32±0.01
	98	EQPLYPQPYQPQ	-0.10	1791.92	0.76±0.00	0.52±0.00	1.03±0.01
Submaxillary gland androgen-regulated protein 3B (SMR3B)							
	98	GPGIFPPPPPQP	0.01	1504.85	1.65±0.01	1.45±0.00	1.11±0.01
	96	GIFPPPPPQP	0.01	1350.77	1.33±0.01	1.79±0.00	0.79±0.01

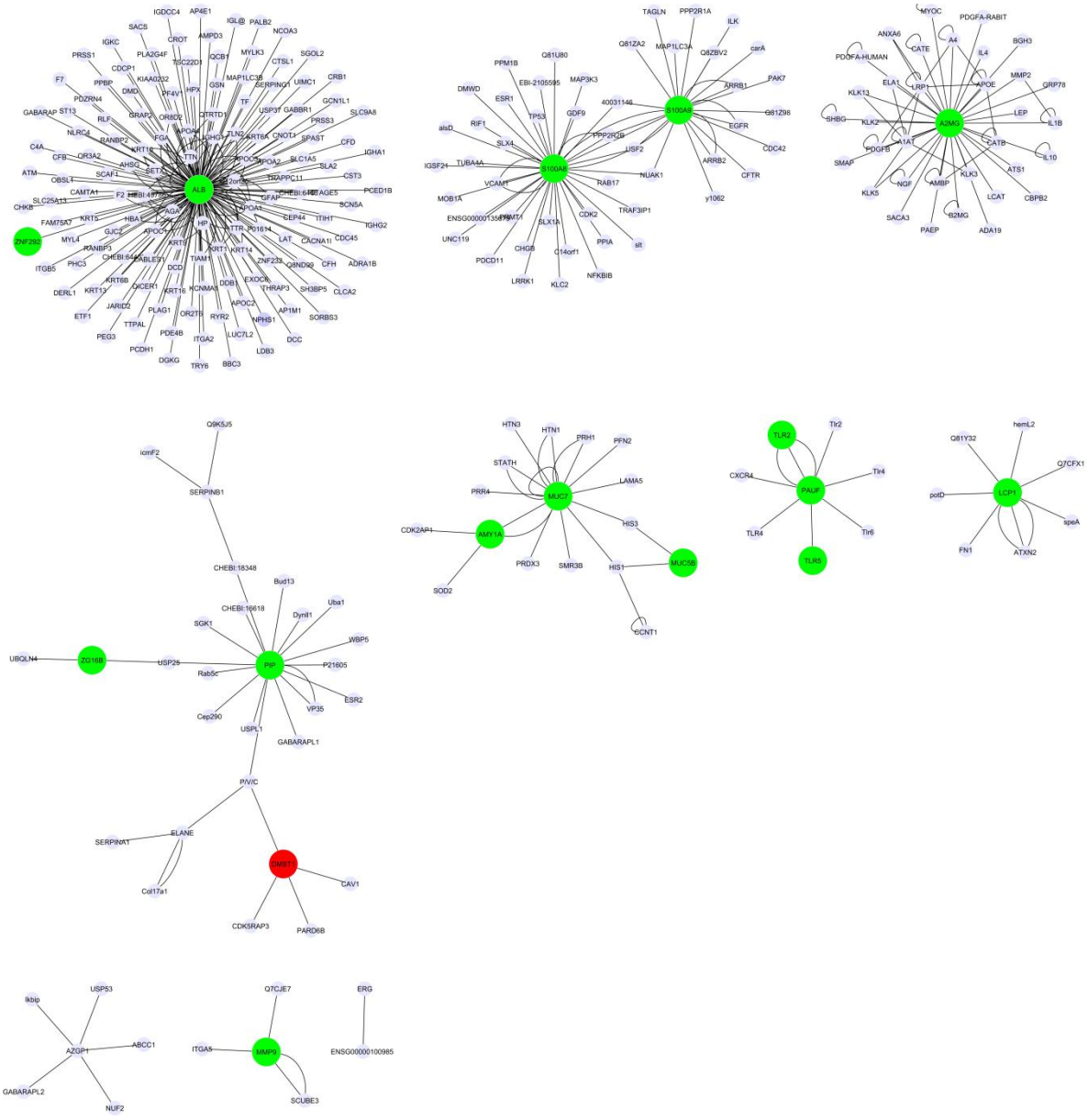
§-iTRAQ4plex; #-Deamidated; Ł-Pro->Val; ♯- Asn->Lys; ł-Carbamyl; #-Deamidated; ♯- Asn->Lys; Ś-His->Pro; Ɣ-Arg->Pro; ¤-Gln->pyro-Glu; ŋ-Pro->Gln;¢-Glu->Gln; ω-Asp->Asn; Ɏ-Asn->Leu;*p<0.05; **p<0.01; ***p<0.001



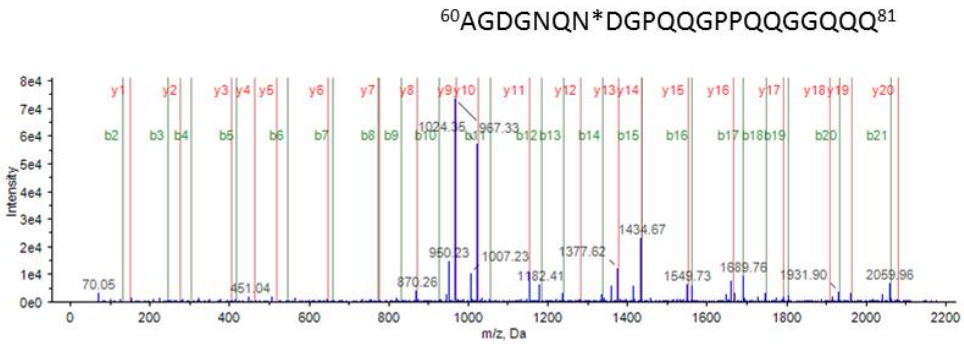
Supplementary Figure S1: Comparison of obtained individual ratio values (log2) for significantly expressed proteins ($p<0.05$) between two independent iTRAQ experiments: T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl.



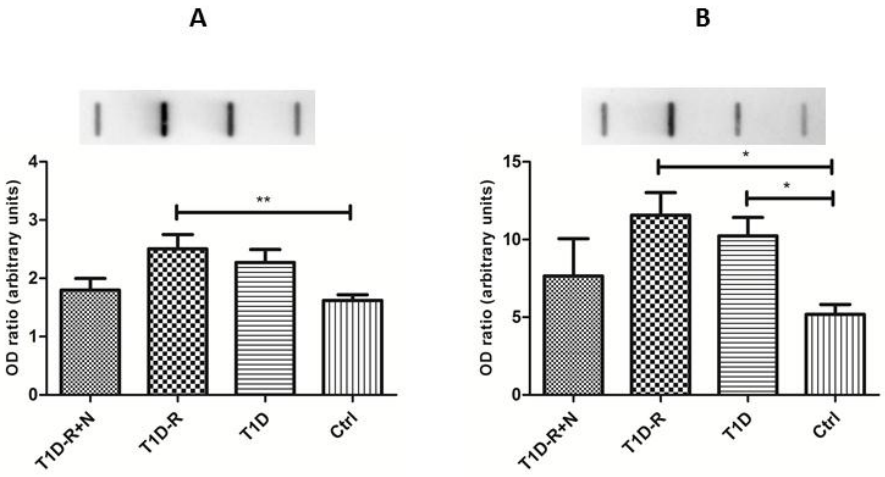
Supplementary Figure S2: Distribution of differentially regulated proteins according to their molecular function (A) and to biological process (B) based on gene ontology annotation.



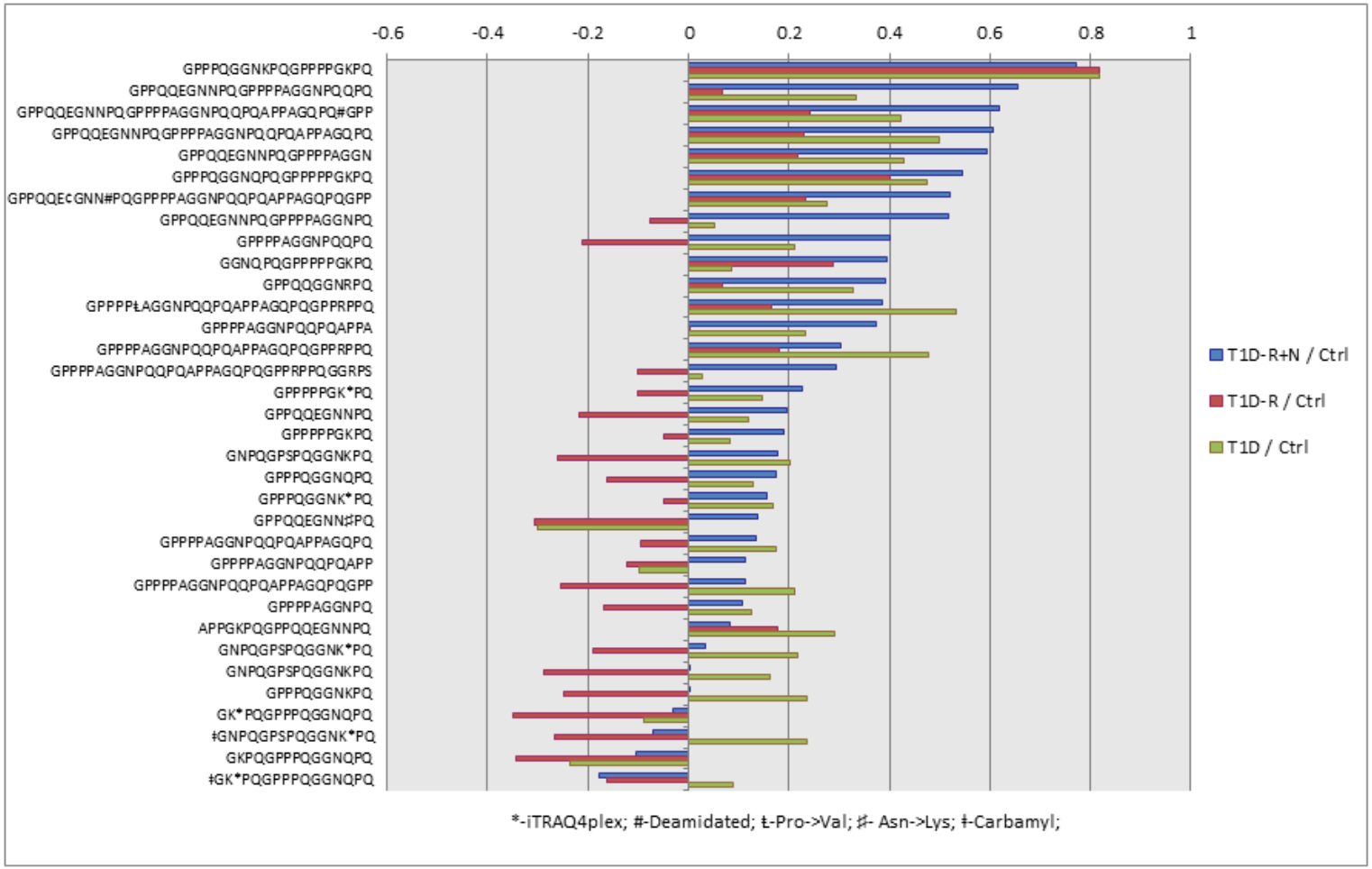
Supplementary Figure S3: This figure shows all DM-regulated GLay clusters of the protein-protein interaction network.



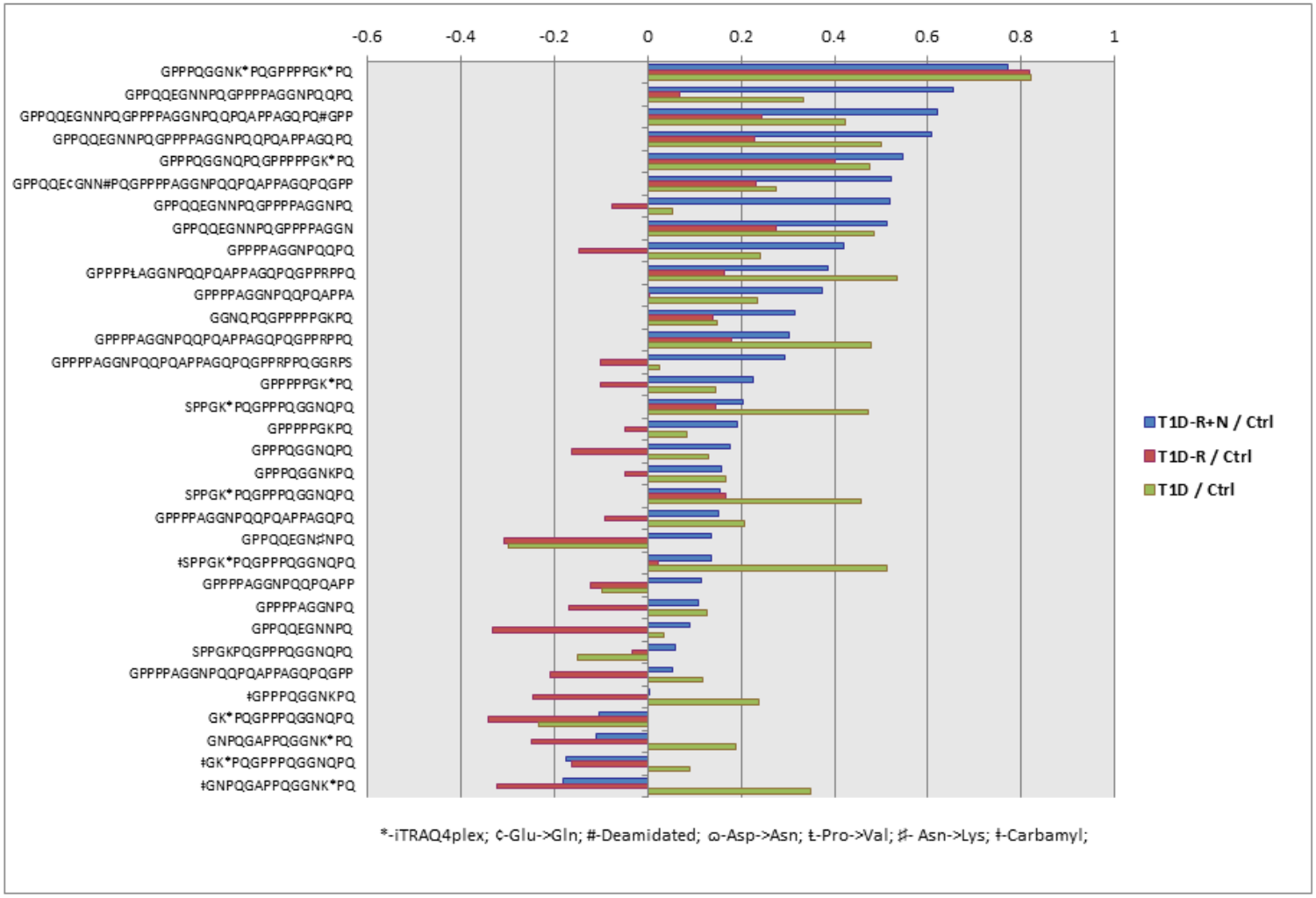
Supplementary Figure S4: MALDI-MS/MS spectra of peptide ^{60}AGDGNQNDGPQ QGPPQQGGQQQ^{81} from PRH2-1 with a m/z of 2204.94, corresponding to the replacement of Asp by Asn in the peptide position 7.



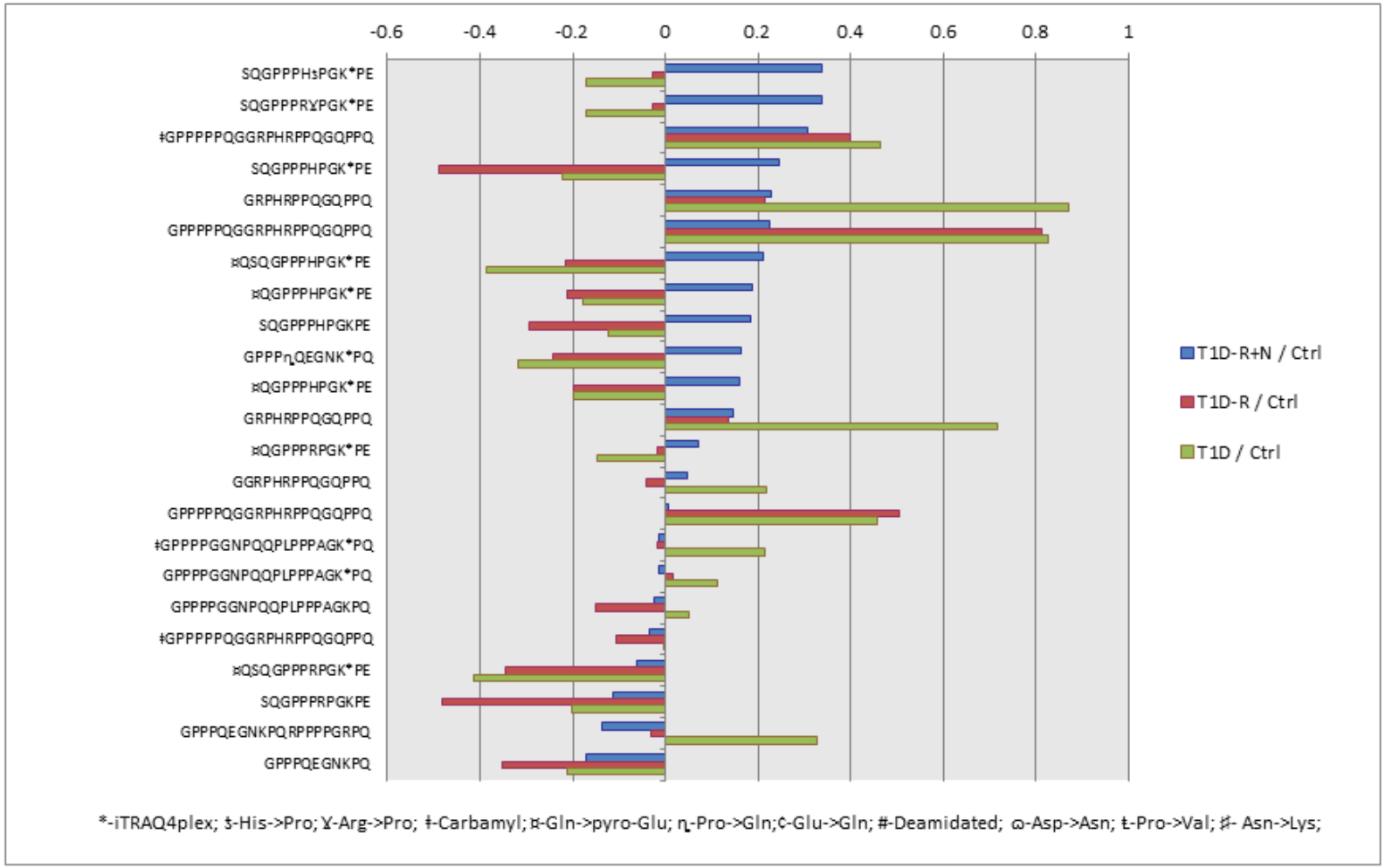
Supplementary Figure S5: Comparative slot-blot analysis of whole saliva glycoproteins (A) and phosphoproteins (B) among groups using specific staining methods (Emerald ProQ and Diamond ProQ, respectively). Representative immunoblot images are presented above the corresponding histograms. (*p<0.05 vs Ctrl).



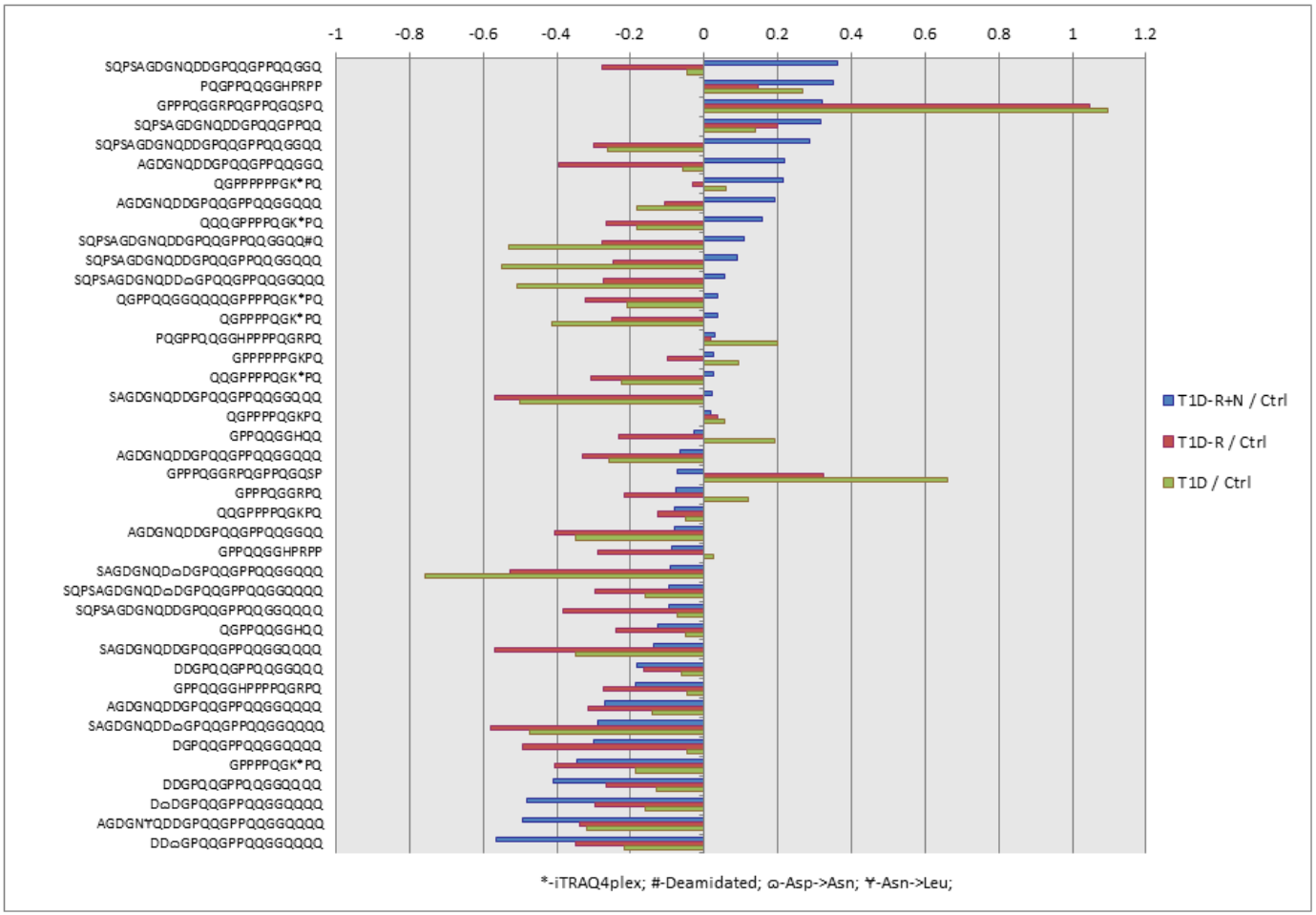
Supplementary Figure S6: Comparison of the log ratio of the relative intensity of the significantly regulated bPRP1 peptides among groups (T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl).



Supplementary Figure S7: Comparison of the log ratio of the relative intensity of the significantly regulated bPRP2 peptides among groups (T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl).



Supplementary Figure S8: Comparison of the log ratio of the relative intensity of the significantly regulated bPRP3 peptides among groups (T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl).



Supplementary Figure S9: Comparison of the log ratio of the relative intensity of the significantly regulated aPRP peptides among groups (T1D-R+N/Ctrl; T1D-R/Ctrl and T1D/Ctrl).