Functional genomics of psoriasis

Alicia Lledo Lara
Hertford College
University of Oxford

A thesis submitted in partial
fulfilment of the requirements for the degree of
Doctor of Philosophy
Trinity Term, 2018

Abstract

Functional genomics of psoriasis

Joe Bloggs, Hertford College, Trinity Term 2014
A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the University of Oxford

This is my abstract...

Acknowledgements

Thank you, thank you, thank you.

Declarations

I declare that unless otherwise stated, all work presented in this thesis is my own. Several aspects of each project relied upon collaboration where part of the work was conducted by others.

Submitted Abstracts

Title **Year**
Authors

Associated Publications

Title
Journal
Authors

Other Publications

Title
Journal
Authors

Contents

List of Figures

List of Tables

Abbreviations

Abbreviation Definition

Chapter 1

Introduction

1.1 Psoriasis and psoriatic arthtritis

Psoriasis (PSO) and psoriatic arthritis (PsA) are considered different common complex disease entities. PSO is a chronic inflammatory skin disease with episodes of relapse and remitance included in the group of inflammatory dermatose diseases (Nestle et al. 2009). On the other hand, PsA is a seronegative, chronic, inflammatory disease within the family of spondyloarthritis (Moll et al. 1973; Coates et al. 2016), usually developed after the manifestation of PSO (**Villanova2016**). PS and PsA have both, similar and different, clinical features, which are likely a reflection of shared and individual genetic loci contributing to the disease development (Variants in RUNX3 contribute to susceptibility to PsA, exhibiting further common ground with ankylosing spondylitis, PsA Immunochip). It is important to understand the commonalities and differences between PSO and PsA at the physiological and genetic level in order to better understand the relevance of the genetic variability in the risk to develop these conditions.

1.1.1 Epidemiology and global impact

PSO represents a serious global problem that currently affects about 100 million people worldwide, including children and adults regardless sex (Organization 2016). Although PSO prevalence presents a very weak correlation

with geographic latitude (Jacobson et al. 2011), it has been reported to vary upon ethnicity. For example, PSO prevalence in adults is comparatively lower among African, African American and Asian (0.4-0.7%) compared to American and Canadian (4.6 and 4.7%, respectively) populations. In the UK, PSO prevalence's estimate ranges between 2-3% and it affects approximately 1.8 million people (Perera et al. 2012).

PsA in the general population ranges between 0.04-1.2% (Perera et al. 2012) but prevalence dramatically increases to 10-30% within PSO cases (**Reich2008**; Gelfand et al. 2005), showing evidence of association between the two diseases. Particularly, in the UK, 14% of the PSO patients develop chronic inflammatory arthritis in the form of PsA at some point of the disease course (Ibrahim et al. 2009). Overall, data suggests an steady increase in both, PSO and PsA, prevalence over time (**Springate2007**; Organization 2016).

Although PSO can be developed at any age, onset of disease seems to have a bimodal distribution strongly influenced by the Human Leukocyte Antigen (HLA) Cw6 (HLA-Cw6), which encodes an allele for one of the genes in the Major Histocompatibility Complex (MHC), involved in antigen presentation and immune cells regulation (Henseler and Christophers 1985). The'early-onset' or Type I is characterised by development of disease around 16-22 and 30-39 years and a greater prevalence for HLA-Cw6 (85.4% of the cases). In contrasts, the late-onset or Type II group manifests disease between 50-60 years old and presents positive HLA-Cw6 only in 14.6% of the cases. This classification based on the age of onset has also distinctive features from a clinical point of view, as they have different severity, relapse frequency and family history.

PSO and PsA also represent a burden for the economy of the countries due to treatment and associated morbidity. For example, in the UK treatment and management of PSO in 2015 ranged between 4,000 to 14,000, before and after requirements of biological therapy, respectively (Burgos-Pol and Dermo 2016).

Regarding PsA, yearly National Health Service (NHS) cost ranges from 11 to 20,782 with a mean of 1,446 per person and significant variation depending on the disease severity (Poole et al. 2010).

1.1.2 PSO and inflammatory dermatoses

The International Classification of Disease 10 (IDC-10) includes more than 1,000 skin or skin-related diseases with heterogeneous prevalence. As a group, it is one of the most prevalent condition that can affect up to 70% of the population, regardless age and geographic location (**ICD-10**). An study in 2010 concluded that skin disease causes a huge burden in the global context of health, being the 4th leading cause of nonfatal burden (**Roderick2014**). The skin is the biggest organ in the human body and it constitutes an effective barrier between the environment and the internal organs. The most external layer, the epidermis, plays a relevant role in the innate and adaptive immunity (Proksch et al. 2008). Therefore, alterations of this barrier due to exogenous (e.g fungus, bacteria) or endogenous (e.g genetic) factors can lead to development of inflammatory dermatose conditions such as PSO, atopic dermatitis (AD), seborrheic dermatitis and cutaneous lupus erythematosus (CLE) (**Johnson-Huang**; **2009**).

The characteristics of the epidermal lesion allows a further classification of the inflammatory dermatoses into non-pustular, pustular and vesiculo-bullous. Due to the great diversity in the type, location and severity the psoriatic lesions can belong to the non-pustular and pustular groups, which demonstrates the heterogeneity of the disease (Perera et al. 2012). As a result, several clinical phenotypes of PSO including vulgaris, guttate, pustular, erythroderma and nail pitting have been defined and it is under debate whether some of those should be considered a different disease entity (**Marrakchi2011**).

1.1.3 PsA and spondyloarthropaties

PsA belong to the family known as spondylarthropaties (SpA) which also includes other subtypes such as ankylosing spondylitis (AS), reactive arthritis (ReA), idiopathic inflammatory bowel disease (IBD) and undifferentiated SpA (). All SpA subtypes are characterised by structural damage (bone formation and erosion) as well as inflammation of joints and extraarticular sites, such as eyes, gut and skin. Additional SpA criteria have led to a reduced classification of SpA into axial and peripheral SpA based on the affected joint (spine/sacroilicac or peripheral) and the presence of extraarticular features (**Runwaleit2001**; **Runwaleit2001**). Familial studies with HLA-B27 positive status have shown manifestation of different SpA forms, such as PSO and IBD, within a single family (**Said-Nahal2000**). Consistently, a transgenic rat model overexpressing the human HLAB27 allele also presented several features of SpA simultaneously (**Hammer1990**). All these observations partially support the hypothesis that SpA subtypes may be a single multifaceted condition with share genetic, immunophatological and structural features, with dynamic phenotypes determined by ubiquitous genetic or environmental factors (**Baeten2013**). Nevertheless, validation of this theory remains challenging due to lack of complete understanding of the cellular and molecular processes that explain SpA pathophysiology. For example, some studies suggest that multiple genetic factors may be involved in the determination of the axial and peripheral arthritis (**Porcher2005**) which could explain some immunopathological differences (**Appel2011**; **Noordenbos2012**).

As a phenotype, PsA can be further subdivided in five clinical groups based on Moll and Wright criteria, : distal, destructive, symmetric, asymmetric and spinal (Moll et al. 1973). These subclasses mainly differed upon the location, number and distribution of the affected joints. Later studies have questioned this method of classification due overlapping of the different subsets and lack of

inclusion of dactylitis (diffuse swelling of a digit), a typical feature of PsA absent in RA (Reich et al. 2009). This phenotypic heterogeneity increases the difficulty in the design and achievement of meaningful outcomes from clinical studies.

1.2 Pathophysiology of psoriasis and psoriatic arthritis

1.2.1 Clinical presentation and diagnosis

The most common form of PSO (approximately 90% of all cases) is plaque PSO vulgaris that manifests with raising well demarcated plaques with, erythema and scaling. The epidermis undergoes an increase in thickness (acanthosis) and vascularisation that causes pinpoint bleeding when the scales are broken (Perera et al. 2012). The plaques vary in thickness and size and tend to be symmetrically distributed, mostly on the elbows, knees, scalp, lumbosacral region, palms and soles (Griffiths and Lancet 2007). A particularly relevant variant of PSO vulgaris is the inverse or flexural, in which the plaques are thinner,non-scaly and shiny and they are located armpits and genitals (Sampogna et al. 2012). The second most common type (10%), PSO guttate, is characterised by acute onset of small droplike papules usually distributed in the trunk and the proximal extremities (Vence et al. 2015). Type I PSO commonly appears in the form of guttate lesions after bacterial infection whilst type II involves spontaneous chronic plaques (Perera et al. 2012). In terms of severity, pustular PSO is the main one potentially life threatening (**Moura2015**).

In PsA the most common manifestation is the symmetric/polyarticular (more than 50%) followed by the asymmetric/oligoarticular (around 30%) PsA, that affects single or few distal interphalangeal (DIP),proximal interphalangeal (PIP) and metatarsal phalangeal (MP) joints, predominantly (**McGonagle2011**; Reich et al. 2009). Clinical or sub-clinical inflammation of the connective tissue

between tendon or ligament and bone (enthesitis) is also found in 35% of the PsA patients (**McGonagle2011**; **Polachek2017**). Axial involvement, mainly spinal, is also present in a quarter of the PsA patients and it increases the risk to develop extraticular features (**Jadon2016**).

As previously mentioned, PsA prevalence is greater in patients with diagnosed PSO and more frequently with type II (**Gu“dh *j“’–o˝nsson2002**). The psoriatic lesions precede joint inflammation in approximately 60-70% of the cases(**Gladman2005**; **McGonagle**; **2011**). Particularly, prevalence of nail lesions is 2.6-fold greater in PSO patients that later develop PsA than in those with uncomplicated PSO (**Moll1976**; Griffiths and Lancet 2007). Similar observations have been made for scalp and intergluteal regions lesions, which together with nail affection constitute a predictive biomarker for development of joint inflammation (**McGonagle**; **2011**). This reinforces the need of appropriate coordination between dermatologists and rheumatologists for an early diagnostic and treatment that could prevent functional joint disability.

Several comorbidities have been associated with PSO and PsA, with comparatively greater prevalence in PsA. For example, intraocular inflammation known as uveitis affects 8% of PsA patients compared to 2% of the PSO ones (**Husted2011**; **Oliveira2015**). Other classic and emerging comorbidities include Inflammatory Bowel Disease (IBD), cardiovascular disease , myocardial infarction (Gelfand et al. 2006),angina and hypertension (Gladman et al. 2009), type II diabetes (T2D) (**Saphiro2007**), metabolic syndrome (**Cohrn20017**) and cancer (Gelfand et al. 2006). PSO and PsA have also important implication in the mental health of the patients and they are associated with an increased prevalence of depression and suicidal ideation (Sampogna et al. 2012).

The diagnosis of PSO and PsA is mainly based in clinical assessment since there is a lack of appropriate biomarkers at early stages of disease (Villanova et al. 2013). PSO skin lesions can be evaluated using the the Psoriasis Area and

Severity Index (PASI). This test quantifies lesional burden weighted by body part based on the amount of affected body surface area and the degree of severity of erythema, induration and scale (**Fredriksson1978**) (Table 1.1). To evaluate PsA, analysis of performance of the previously mentioned Moll and Wright criteria together with additional ones such as the Vasey and Espinoza, Gladman et al. and McGonagle led to the confirguration of the Classification Criteria for Psoriatic Arthritis (CASPAR), the most widely used. It requires the patient displaying inflammatory arthritis, enthesitis, and/or spondylitis and 3 points from a list of associated elements (Table 1.2.1) (**Taylor2006**). Another composite measure commonly used to evaluate treatment efficacy for PsA is the PsA Response Criteria (PSARC) based on the number of tender joints (TJC) and swollen joints (SJC) over 68 and 66, respectively, as well as a physician global assessment based on a short questionnaire (**Philipp2011**; **Clegg1996**)

Feature	Scoring scale
Body location	Head and neck, upper limbs, trunk and lower limbs
Intensity	Redness, thickness and scaling
Severity	Absent, mild, moderate, severe or very severe
Affected area	Nil (0), 1-9%, 10-29%, 30-49%, 50-69%, 70-89% or 90-100%

Table 1.1:

1.2.2 Aetiology

PSO and PsA are complex chronic inflammatory diseases where genetically predisposed individuals and dysregulation of the immune response after exposure to a particular environmental trigger initiate disease (Figure). Although there are some studies regarding the self-tolerance and presence of

A patient must have inflammatory articular disease (joint, spine, or enthesial) with 3 points from 5 categories

1. PSO a. Current skin or scalp disease
b. History of PSO
c. Family history of PSO
2. Psoriatic nail involvement Typical psoriatic nail distrophy
3. A negative test for RF Using preferrably by enzyme-linked immunosorbent assay (EMSA)
4. Dactylitis a. Swelling of an entire finger
b. History of dactylitis
5. Radiologic evidence of juxtaarticular new bone formation Ossification near joint margins

A patient must have inflammatory articular disease (joint, spine, or enthesial) w	
PSO	a. Current skin b. Histo c. Family h
Psoriatic nail involvement	Typical psoriat
A negative test for RF	Using preferrably by enzyme-lin
Dactylitis	a. Swelling of b. History
Radiologic evidence of juxtaarticular new bone formation	Ossification n

autoantigenes as disease trigger (Lande et al. 2007), the autoimmune aetiology of PSO is still under debate.

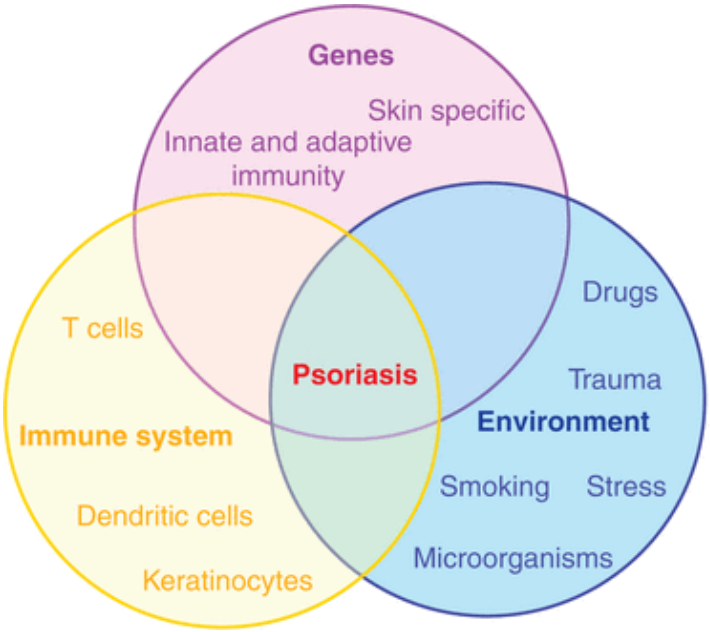


Figure 1.1: Figure adapted from (Meglio2014)

Process that leads to disease. Maybe I can talk about the skin and how it leads to the lessions,summary of how disease occurs and about autoimmunity. Same for PsA Include environmental risk factors

The etiology of psoriasis remains unclear, although there is evidence for genetic predisposition (4). The role of the immune system in psoriasis causation is also a major topic of research. Although there is a suggestion that psoriasis could be an autoimmune disease, no autoantigen that could be responsible has been defined yet. Psoriasis can also be provoked by external and internal triggers, including mild trauma, sunburn, infections, systemic drugs and stress (5).

Cell types involved in pathophysiology

Therapeutic intervention and diagnosis

GWAS and functional genomics of psoriasis and other complex diseases

Epigenetics and gene expression

Understanding the chromatin landscape Chromatin accessibility Histones modifications Understanding the chromatin landscape Transcription factor occupancy Chromatin interaction

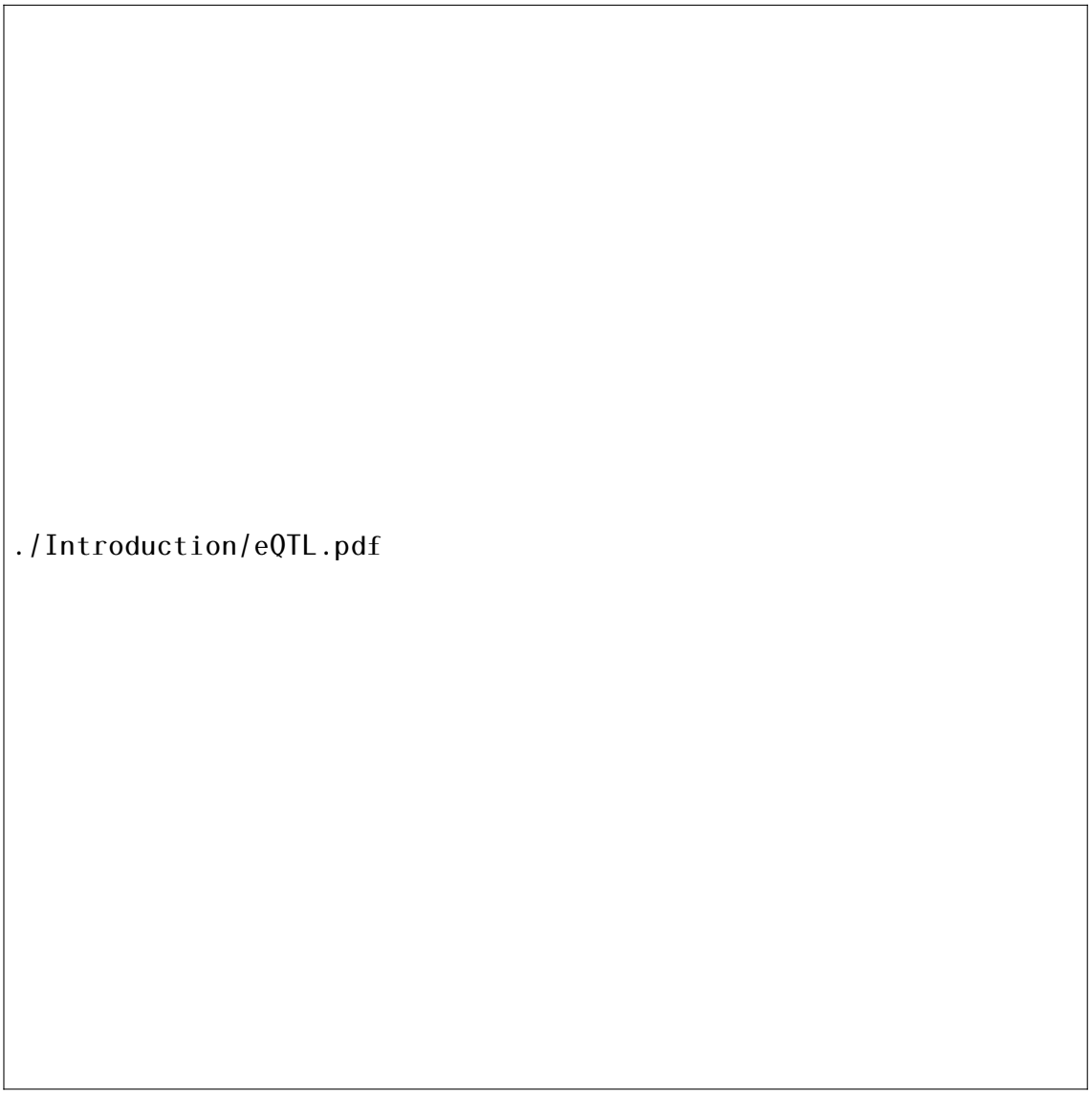


Figure 1.2: Effect of regulatory variants on expression levels of genes. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Genetics (**Cheung2009**), copyright 2009. The effect of local (*cis*) or distal (*trans*) regulatory variants on levels of gene expression.

1.3 Sepsis

Sepsis is defined as the systemic inflammatory response to the presence of an infection and is classified as severe when associated with organ dysfunction, hypoperfusion abnormality or sepsis-induced hypotension. Systemic inflammatory response syndrome (SIRS) is used to describe the inflammatory response and it includes at least two of the clinical manifestations

detailed in Table 1.2 (**Bone1992**). However this definition is limited in that the microbiological basis of the response is not considered and there is no graduation of severity. In the UK, severe sepsis accounts for 27% of ICU admissions (**Padkin2003**) and from a number of studies in both the UK and America, mortality has been estimated to be between 28% and 50% (**Angus2001**; **Padkin2003**; **Sands1997**; **Zeni1997**).

Table 1.2: The clinical manifestations of SIRS (Bone1992). SIRS is diagnosed in patients with at least two of these clinical manifestations.

SIRS clinical manifestations
Body temperature >38°C
Heart rate >90 beats per minute
Respiratory rate >20 breaths per minute or hyperventilation as indicated by a $PACO_2$ of <32 mm Hg
An alteration in the white blood cell count (count >12,000/cu mm or <400/cu mm or the presence of >10% immature neutrophils)

1.4 Common variable immune deficiency disorders

Primary immunodeficiencies (PIDs) are a group of rare disorders that result from a failure in the immune system and CVID are the most frequently encountered PID in the clinic (**Park2008**). CVID are a group of diseases in which insufficient quantity and quality of immunoglobulin usually leads to susceptibility to recurrent bacterial infections, mainly of the respiratory and gastrointestinal tracts (**Chapel2009**). An immunodeficiency is recognized in most CVID patients in the second, third or fourth decade of life and the first diagnostic criteria were published in 1999 (Table 1.3) (**Conley1999**). These

criteria have been used since then with slight variations for example, the minimum age of presentation is often increased to 4 years in order to avoid infants with immune defects which cause B cell differentiation and class-switch disorders (**Chapel2008**).

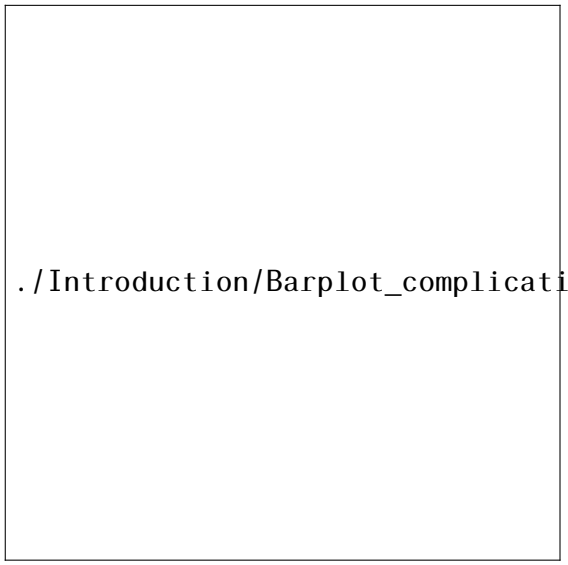
Table 1.3: CVID diagnosis criteria. Individuals fulfilling each of the criteria are diagnosed with CVID (**Conley1999**). SD = standard deviations.

Criteria for CVID diagnosis
Male or female patient >2 years of age
Serum IgG and IgA at least 2 SD below the mean for age
IgM is present or absent
Poor response to vaccines
Absent isohemagglutinins
Defined causes of hypogammaglobulinemia have been excluded
Normal or low B cell numbers

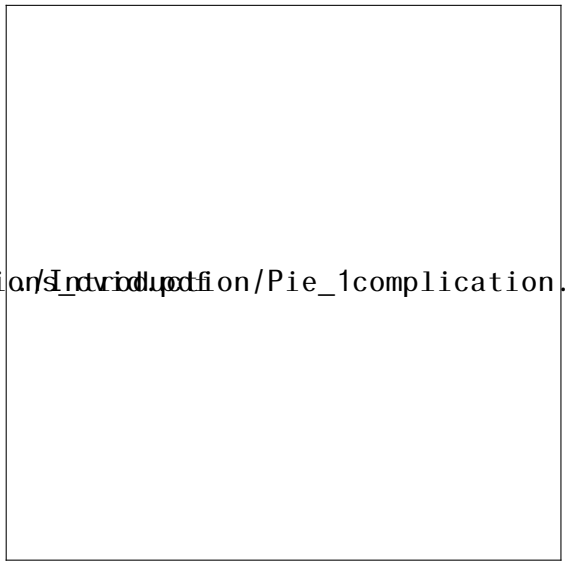
1.4.1 Clinical complications

The diagnosis of CVID is made by excluding known disorders of B cell failure for example B cell differentiation defects resulting in absent B cells, activation-induced cytidine deaminase (AID) and uracil-DNA glycosylase (UNG) deficiencies affecting B cell function and T cell switching pathways (**Chapel2008**). This results in a heterogeneous group of individuals with widely different clinical features and additional complications.

Within cohorts of CVID patients, beyond bacterial infections, the main clinical complications observed include autoimmunity, lymphocytic infiltration, enteropathy and malignancy (Figure 1.3). Patients with at least one clinical complication have a much poorer survival rate compared to those with an Infections Only phenotype (**Chapel2008**).



(a) Number of complications



(b) One complication

(c) Two complications

(d) Three complications

Figure 1.3: Nature and number of CVID complications. a) Number of complications observed in each individual. Individuals with no complications are referred to as infections only. b) Distribution of complications in patients with one complication. c) Distribution of complications in patients with two complications. d) Distribution of complications in patients with three complications. (**Chapel2008**)

Bibliography

Burgos-Pol, R. and Dermo, Martnez-Sesmero-J. M. (2016). "The cost of psoriasis and psoriatic arthritis in 5 European countries: a systematic review". *Actas Dermo*. URL: http://www.sciencedirect.com/science/article/pii/S1578219016301524.

Coates, L. C., FitzGerald, O., Helliwell, P. S., and and, Paul-C. in arthritis (2016). "Psoriasis, psoriatic arthritis, and rheumatoid arthritis: Is all inflammation the same?" *Seminars in arthritis and*. URL: http://www.sciencedirect.com/science/article/pii/S0049017216300646.

Gelfand, J. M., Gladman, D. D., Mease, P. J., and Academy, Smith-N. (2005). "Epidemiology of psoriatic arthritis in the population of the United States". *the American Academy*. URL: http://www.sciencedirect.com/science/article/pii/S0190962205010108.

Gelfand, J. M., Neimann, A. L., Shin, D. B., and Jama, Wang-X. (2006). "Risk of myocardial infarction in patients with psoriasis". *Jama*. URL: http://jamanetwork.com/journals/jama/fullarticle/203598.

Gladman, D. D., Ang, M., Su, L., and the, B. D. M. of (2009). "Cardiovascular morbidity in psoriatic arthritis". *Annals of the*. DOI: 10.1136/ard.2008.094839. URL: http://dx.doi.org/10.1136/ard.2008.094839.

Griffiths, C. E. M. and Lancet, Jnwn (2007). "Pathogenesis and clinical features of psoriasis". *The Lancet*. URL: http://www.sciencedirect.com/science/article/pii/S0140673607611283.

Henseler, T. and Christophers, E. (1985). "Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris". *J Am Acad Dermatol* 13: pp. 450–6. ISSN: 0190-9622 (Print) 0190-9622.

Ibrahim, G., Waxman, R., and Helliwell, P. S. (2009). "The prevalence of psoriatic arthritis in people with psoriasis". *Arthritis Rheum* 61: pp. 1373–8. ISSN: 0004-3591 (Print) 0004-3591. DOI: 10.1002/art.24608.

Jacobson, Christine C., Kumar, Sandeep, and Kimball, Alexa B. (2011). "Latitude and psoriasis prevalence". *Journal of the American Academy of Dermatology* 65: pp. 870–873. ISSN: 0190-9622. DOI: 10.1016/j.jaad.2009.05.047. URL: http://dx.doi.org/10.1016/j.jaad.2009.05.047.

Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y. H., Homey, B., Cao, W., Su, B., Nestle, F. O., Zal, T., et al. (2007). "Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide". *Nature* 449: pp. 564–9. ISSN: 0028-0836. DOI: 10.1038/nature06116. URL: http://dx.doi.org/10.1038/nature06116.

Moll, J. M. H., in, arthritis, and rheumatism, Wright-V. (1973). "Psoriatic arthritis". *Seminars in arthritis and rheumatism*. URL: http://www.sciencedirect.com/science/article/pii/0049017273900358.

Nestle, Frank O., Kaplan, Daniel H., and Barker, Jonathan (2009). "Psoriasis". *New England Journal of Medicine* 361: pp. 496–509. DOI: doi : 10 . 1056 / NEJMra0804595. URL: http : / / www . nejm . org / doi / full / 10 . 1056 / NEJMra0804595.

Organization, World Health (2016). *Global report on psoriasis*. Report.

Perera, G. K., Di Meglio, P., and Nestle, F. O. (2012). "Psoriasis". *Annu Rev Pathol* 7: pp. 385–422. ISSN: 1553-4006. DOI: 10 . 1146 / annurev - pathol - 011811 - 132448.

Poole, C. D., Lebmeier, M., Ara, R., and Rheumatology, Rafia-R. (2010). "Estimation of health care costs as a function of disease severity in people with psoriatic arthritis in the UK". *Rheumatology*. DOI: 10 . 1093 / rheumatology / keq182. URL: http : / / dx . doi . org / 10 . 1093 / rheumatology/keq182.

Proksch, E., Brandner, J. M., and Jensen, J. M. (2008). "The skin: an indispensable barrier". *Exp Dermatol* 17: pp. 1063–72. ISSN: 0906-6705.

Reich, K., Krger, K., and Dermatology, Mssner-R. of (2009). "Epidemiology and clinical pattern of psoriatic arthritis in Germany: a prospective interdisciplinary epidemiological study of 1511 patients with plaquetype psoriasis". *Journal of Dermatology*. DOI: 10 . 1111 / j . 1365 - 2133 . 2008 . 09023 . x. URL: http://dx.doi.org/10.1111/j.1365-2133.2008.09023.x.

Sampogna, Francesca, Tabolli, Stefano, and Abeni, Damiano (2012). "Living with psoriasis: prevalence of shame, anger, worry, and problems in daily activities and social life". *Acta dermato-venereologica* 92: pp. 299–303. DOI: 10 . 2340 / 00015555 - 1273. URL: http : / / dx . doi . org / 10 . 2340 / 00015555 - 1273.

Vence, L., Schmitt, A., and Medical, Meadows-C. E. (2015). "Recognizing guttate psoriasis and initiating appropriate treatment". *West Virginia Medical*. URL: http : / / go . galegroup . com / ps / i . do ? id = GALE %7CA422778063 & sid = googleScholar & v = 2 . 1 & it = r & linkaccess = fulltext & issn = 00433284 & p = AONE & sw = w.

Villanova, Federica, Meglio, Paola, and Nestle, Frank O. (2013). "Biomarkers in psoriasis and psoriatic arthritis". *Annals of the rheumatic diseases* 72. DOI: 10 . 1136 / annrheumdis - 2012 - 203037. URL: http : / / dx . doi . org / 10 . 1136 / annrheumdis - 2012 - 203037.