

## Online Appendix 1

| <i>Abbreviation</i> | <i>Description</i>                      | <i>Unit</i> | <i>Availability</i> | <i>SOM input</i> |
|---------------------|-----------------------------------------|-------------|---------------------|------------------|
| Adipon              | adiponectin                             | mg/l        | 58%                 | yes              |
| AHT                 | anti-hypertensive treatment             | -           | 100%                | -                |
| ApoAI               | serum apolipoprotein A-I                | mg/dl       | 93%                 | yes              |
| ApoAII              | serum apolipoprotein A-II               | mg/dl       | 89%                 | no               |
| ApoB                | serum apolipoprotein B                  | mg/dl       | 93%                 | yes              |
| Chol                | total cholesterol                       | mmol/l      | 97%                 | yes              |
| CPept               | C-peptide                               | pmol/l      | 76%                 | no               |
| CRP                 | C-reactive protein                      | mg/l        | 82%                 | yes              |
| DBP                 | diastolic blood pressure                | mmHg        | 98%                 | -                |
| DKD                 | diabetic kidney disease*                | -           | 90%                 | -                |
| DMDur               | duration of type 1 diabetes             | years       | 100%                | -                |
| DRP                 | diabetic retinal disease                | -           | 99%                 | -                |
| A1C                 | glycosylated hemoglobin A <sub>1c</sub> | %           | 95%                 | yes              |
| HDL <sub>2</sub> C  | high-density lipoprotein 2 cholesterol  | mmol/l      | 92%                 | yes              |
| HDL <sub>3</sub> C  | high-density lipoprotein 3 cholesterol  | mmol/l      | 92%                 | no               |
| LDLC                | LDL cholesterol (Friedewald)            | mmol/l      | 96%                 | no               |
| MBL                 | mannan binding lectin                   | mg/l        | 59%                 | yes              |
| MetS                | metabolic syndrome                      | -           | 94%                 | -                |
| SBP                 | systolic blood pressure                 | mmHg        | 98%                 | -                |
| sCreat              | serum creatinine                        | μmol/l      | 96%                 | yes              |
| sK                  | serum potassium                         | mmol/l      | 55%                 | yes              |
| sNa                 | serum sodium                            | mmol/l      | 55%                 | yes              |
| TG                  | triglyceride concentration              | mmol/l      | 97%                 | yes              |
| 24h-uAlb            | 24h-urine albumin excretion             | mg          | 80%                 | yes              |
| 24h-uCreat          | 24h-urine creatinine excretion          | mmol        | 66%                 | yes              |
| 24h-uK              | 24h-urine potassium excretion           | mmol        | 62%                 | no               |
| 24h-uNa             | 24h-urine sodium excretion              | mmol        | 62%                 | no               |
| 24h-uUrea           | 24h-urine urea excretion                | mmol        | 61%                 | no               |

Only biochemical variables were included in the SOM input data. Furthermore, variables that were derived mathematically (Friedewald LDLC) or were strongly collinear with the other inputs (HDL<sub>3</sub>C, ApoAII, 24h-uK, 24h-uNa, 24h-uUrea) were excluded (the low availability of the urine data caused additional artifacts in the analyses). C-peptide was close to the detection limit for almost all patients and was also excluded. SOM models that included all the biochemical variables were nevertheless tested to ensure that the model pruning did not reduce statistical sensitivity. Before analysis, the input data was rank-transformed for men and women separately.

The robustness of the analyses was tested by removing patients with low availability of data; the results for the more complete subsets were consistent with the full dataset (not shown). In theory, the SOM algorithm is able to automatically ignore any missing values, but due to the PCA initialization (illustrated in Online Appendix 2), the preprocessed data was imputed by least-squares linear regression; this also ensured that the missing values did not cause any non-linear artifacts.

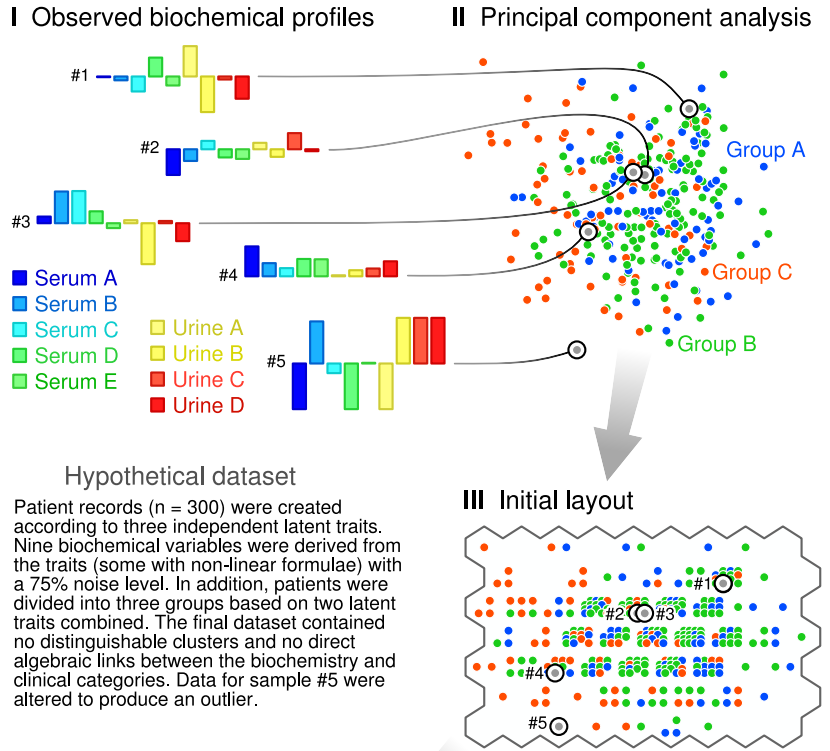
\*All patients were reviewed for DKD status, but 10% of the individuals could not be classified due to inconclusive clinical data.

# Self-organizing map

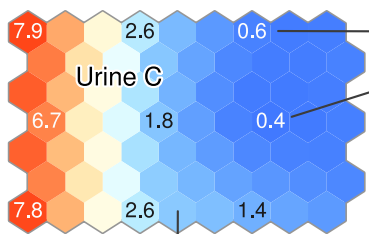
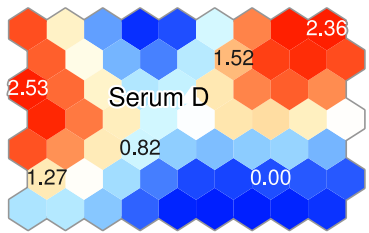
## MeliKerion – free software for SOM analysis

The integration of numerous sources of biochemical and clinical materials sets a challenge to the researchers who are striving for a deeper insight into complex pathogenic mechanisms. Classical reductionist approaches often overlook non-linearity and rely on univariate diagnostic criteria as targets of inference, which may prevent the detection of complex multi-dimensional interactions. Unsupervised analysis of the regularities within a dataset is the first step in transforming the measurements into usable knowledge, but estimating the statistical significance of the observed patterns is often difficult.

MeliKerion is an implementation of the Kohonen self-organizing neural network algorithm, and was designed for unsupervised analysis of clinical materials. A typical process flow is depicted on the right for a small dataset with diffuse phenotypical boundaries. In this simulated case, the emphasis is on the biochemical profiles, thus the first step is to preprocess the measurement data so that the variables become comparable in scale and mean value (Stage I). Next, linear decomposition (Stage II) is applied to create an initial map layout of patients (Stage III). The final layout is achieved after several iterations of a batch version of the Kohonen algorithm (Stage IV). Once the map is complete, the full dataset can be visualized based on the biochemical profiles, with significance estimates for clinical variables.

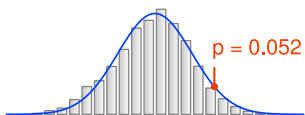


### Internal component planes

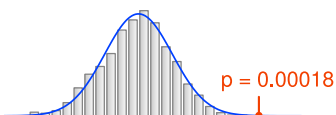


The biochemical data are transformed to original scale and mean value before coloring the planes.

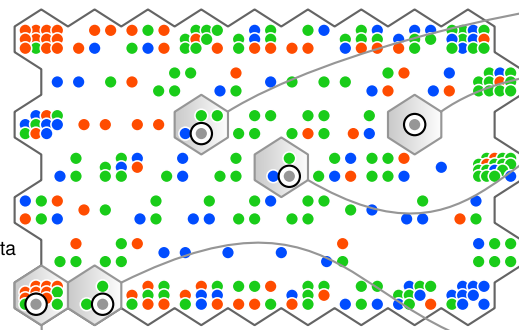
The dynamic range of the pseudo-colors is proportional to the expected random fluctuation; the colors are thus comparable between the component planes.



P-values for the observed regional variability can be estimated by simulating a situation where the biochemistry is not related to the clinical data (null hypothesis). If the observed statistic is very different from the null distribution, the observation is not likely to have occurred by chance. Here, 1000 such simulations were performed for each clinical group.



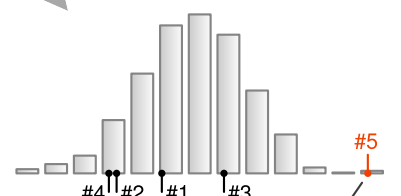
### IV Layout after self-organizing algorithm



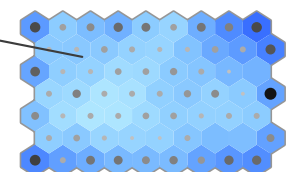
### Model profiles

On each map unit, the neural network creates a model phenotype (grey) and together the models describe the recurring statistical features in the dataset. A patient's place on the map is set by the model that best matches the observed profile (colors).

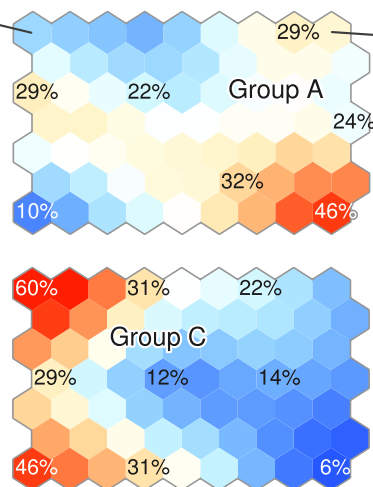
### Map quality



The error between an observed biochemical profile and the respective model reveals outliers.



### External component planes with statistical significance estimates

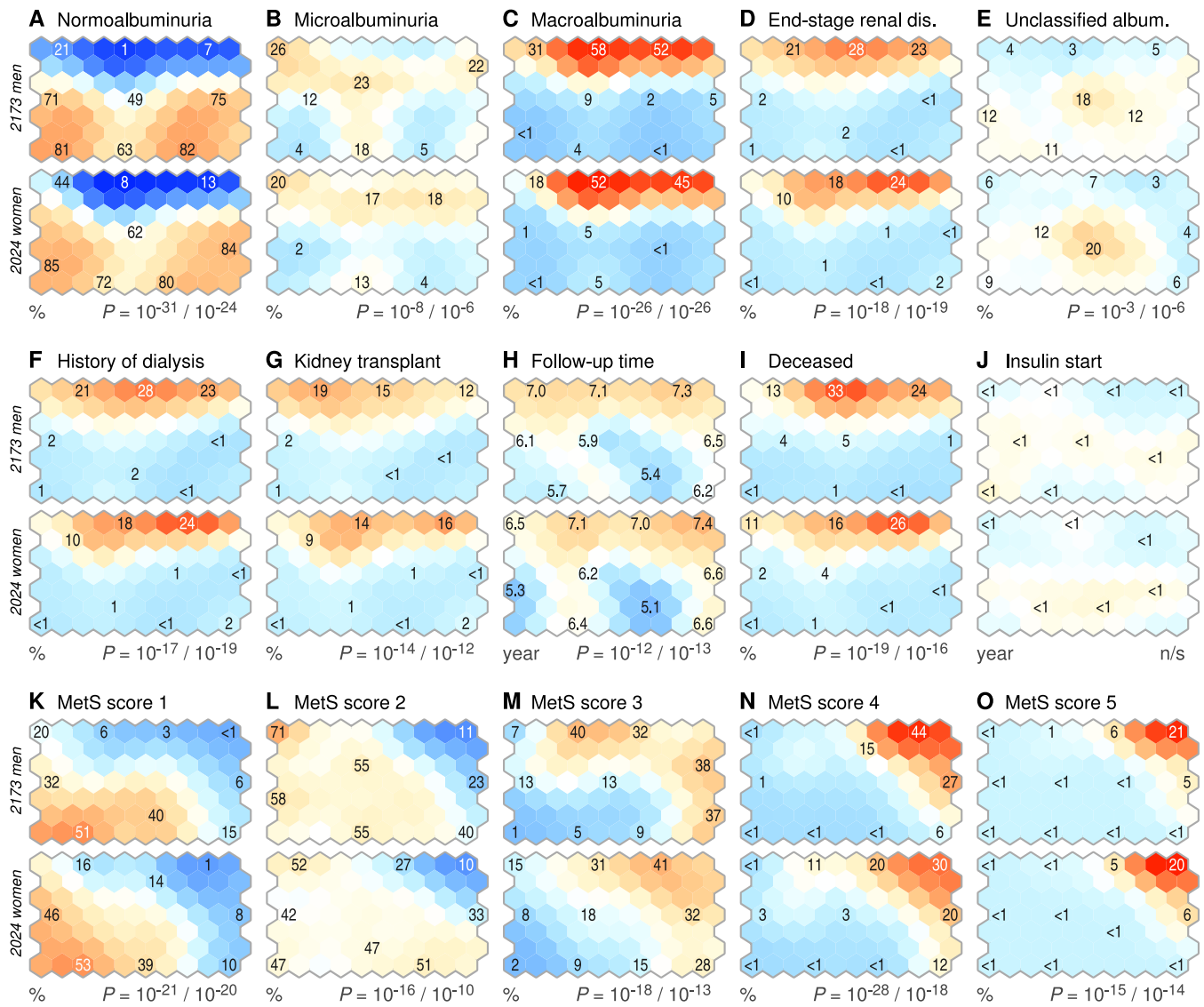


For categorical and binary variables, the map can be colored with respect to the demographic proportions on the hexagonal units.

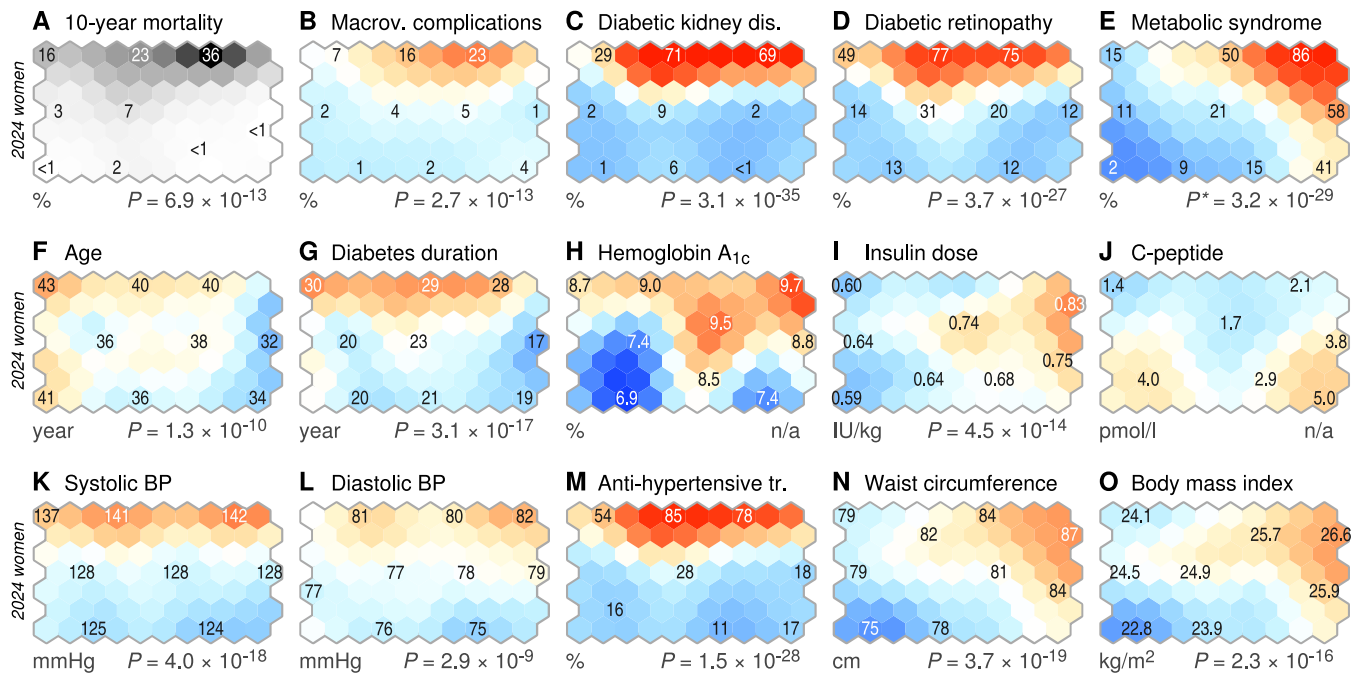
Clusters in the data can be detected by estimating the rate of change of the model profiles between adjacent map units. Also, the areas between clusters would be void of individuals. In this case, the dataset was diffuse, hence the models change smoothly when moving across the map.

More details can be found in Supplement 1 of Mäkinen V-P et al.: <sup>1</sup>H NMR metabonomics approach to the disease continuum of diabetic complications and premature death. *Molecular Systems Biology* 4:167, 2008.

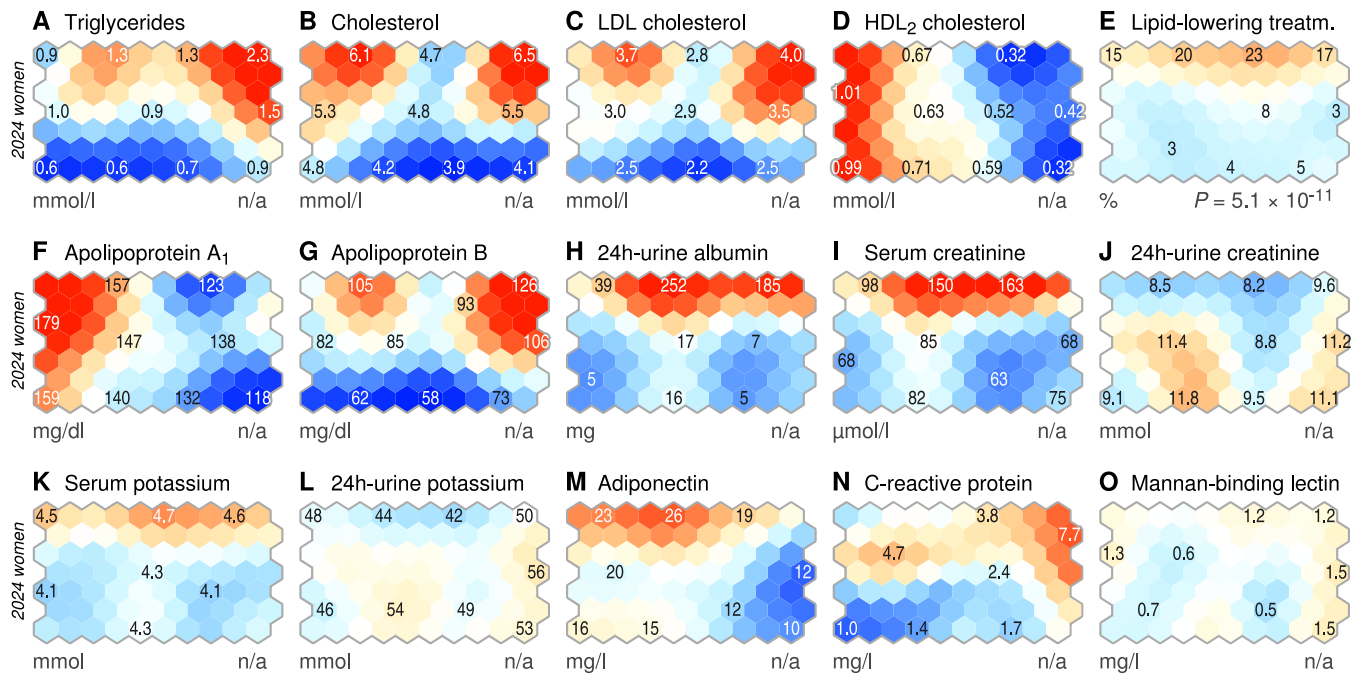
The MeliKerion is available at [www.computationalmedicine.fi/software](http://www.computationalmedicine.fi/software).



Online Appendix 3

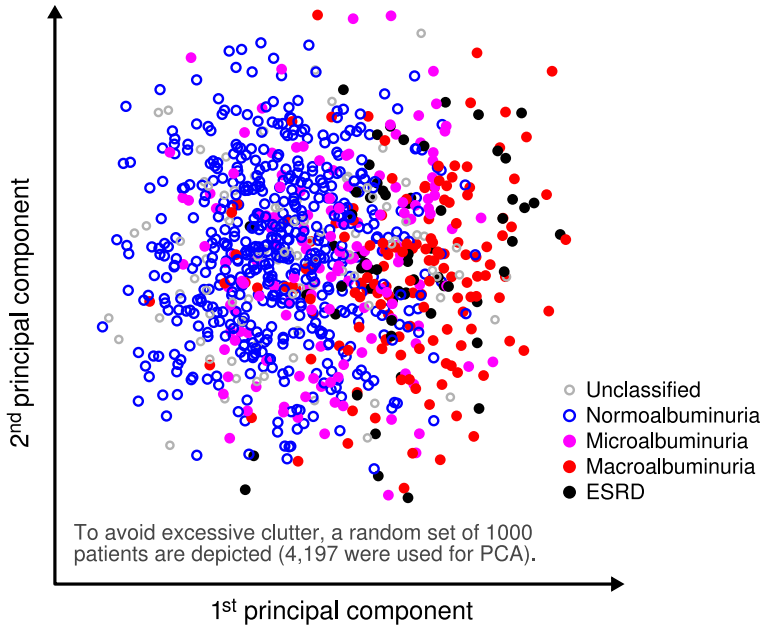


Online Appendix 4

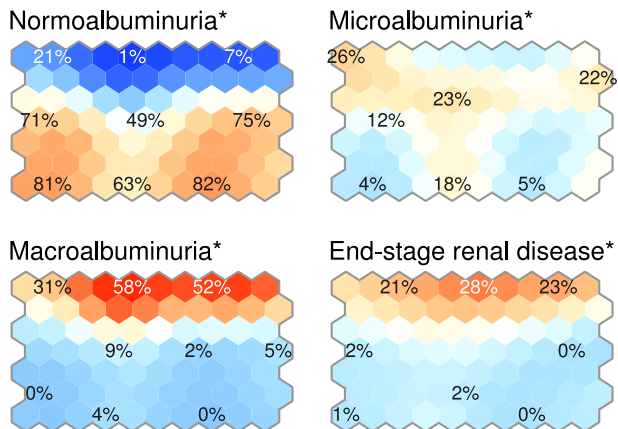
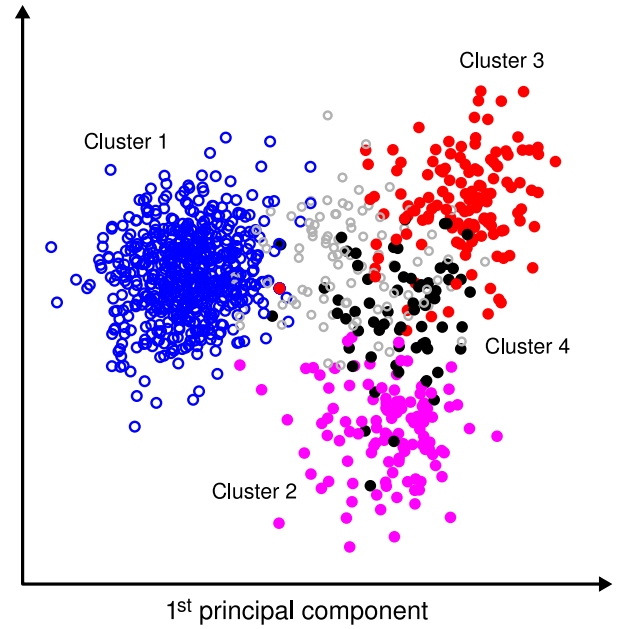


Online Appendix 5

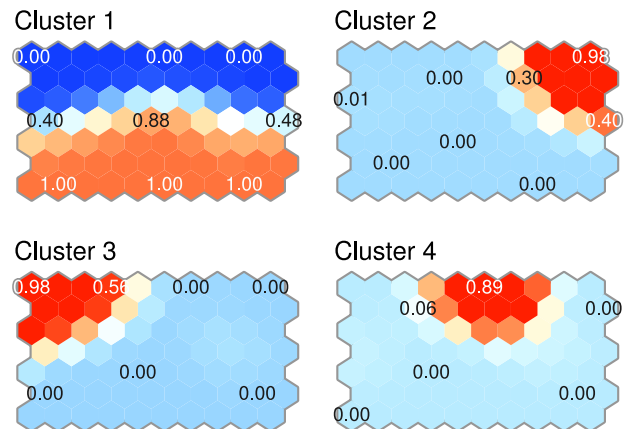
### The FinnDiane dataset



### Simulated data with clusters

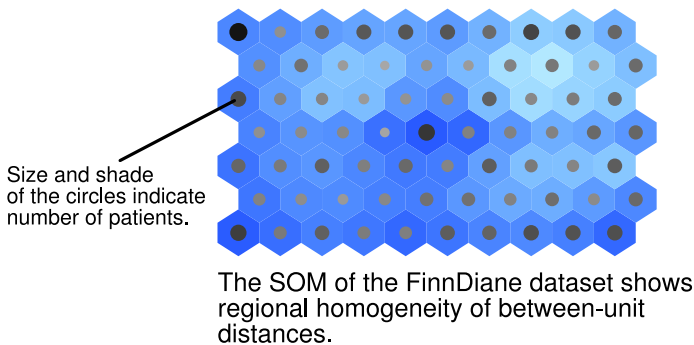


\*Men only, women are shown in Online Appendix 2.

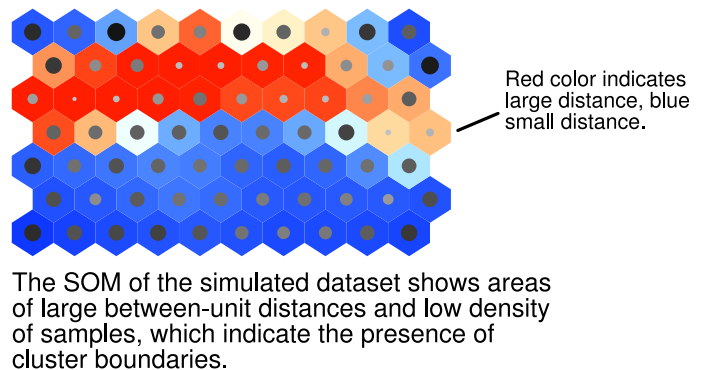


### Euclidean distance in data space between map units

FinnDiane



Simulated



## Online Appendix 7

# The Finnish Diabetic Nephropathy Study Centers

|                                                                        |                                                                                                |                                      |                                                                                        |
|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------------|
| Anjalankoski health care center                                        | Sirpa Koivula<br>Tarja Uggeldahl                                                               | Helsinki Herttoniemi Hospital        | Markku Saraheimo<br>Kerstin Wickström                                                  |
| Espoo health care center, Espoonlahti                                  | Anita Nikkola<br>Eila Ritola                                                                   | Helsinki University Central Hospital | Paula Nikkanen<br>Pirkko Salmi                                                         |
| Espoo health care center, Samaria<br>Espoo health care center, Tapiola | Eija Oukko-Ruponen<br>Hanna Saarinen<br>Merja Kataja-Tuomola<br>Marjatta Niska                 | Hyvinkää health care center          | Sisko Klemetti<br>Teija Nyandoto<br>Eija Rontu<br>Susanna Satuli-Autere                |
| Espoo health care center, Viherlaakso                                  | Anne Lyytinen<br>Satu Raumavirta-Koivisto<br>Marjo Kovanen                                     | Iisalmi Hospital                     | Eija Toivanen<br>Jaana Hyytiäinen                                                      |
| Etelä-Karjala Central Hospital                                         | Tuula Ensala<br>Esko Hussi<br>Jukka Toivonen<br>Tuula Matikainen<br>Raimo Vanamo<br>Helga Hasu | Jokilaakso Hospital                  | Tuula Laitinen<br>Jukka Lagerstam<br>Suvimarja Aranko<br>Tarmo Leppälä<br>Suvi Ervasti |
| Heinola health care center                                             | Päivi Hentunen<br>Jukka Lagerstam                                                              | Jorvi Hospital                       | Ritva Kauppinen-Mäkelin<br>Arto Kuusisto<br>Katriina Nikkilä<br>Leena Pekkonen         |
| Helsinki health care center, Puistola                                  | Kari Pohjola<br>Heli Silvennoinen<br>Tiina Simonen<br>Hannele Kari                             | Jyväskylä health care center         | Kirsti Nuorva<br>Marja Tiihonen<br>Tuula Kinnunen<br>Liisa Nikulainen                  |
| Helsinki health care center, Suutarila                                 | Anja Kaprio<br>Birgitta Rantaeskola                                                            | Järvenpää health care center         | Tuula Salokangas<br>Silja Jokelainen<br>Paula Kemppainen<br>Anna-Maria Mankinen        |
| Helsinki health care center, Töölö                                     | Jari Haaga<br>Pirjo Kääriäinen<br>Anna-Liisa Pietiläinen                                       | Kainuu Central Hospital              | Marja Sankari                                                                          |



|                                  |                                                                                                                                                                                   |                                                     |                                                                                                                                                                                                                 |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Kanta-Häme Central Hospital      | Tuula Myllykangas<br>Pirjo Kinnunen<br>Anja Orvola<br>Tapani Salonen<br>Annukka Vähänen                                                                                           | Kuopio University Central Hospital                  | Erja Huttunen<br>Risto Ikäheimo<br>Päivi Kiljander<br>Markku Laakso<br>Timo Lakka                                                                                                                               |
| Kerava health care center        | Helene Stuckey<br>Paula Suominen                                                                                                                                                  |                                                     | Leena Moilanen<br>Leo Niskanen                                                                                                                                                                                  |
| Keski-Pohjanmaa Central Hospital | Stefan Anderson<br>Beatrice Asplund<br>Ulrika Byskata<br>Ingvor Byskata<br>Toini Virkkala<br>Päivi Liedes                                                                         |                                                     | Ulla Tuovinen<br>Ilkka Vauhkonen<br>Erkki Voutilainen<br>Pauli Karhapää<br>Päivi Kekäläinen<br>Erkki Lampainen                                                                                                  |
| Keski-Suomi Central Hospital     | Terje Forslund<br>Pirkko Koskiaho<br>Marianne Laukkanen<br>Juha Saltevo<br>Marja Tiihonen<br>Pirkko Koskiaho<br>Anne Halonen<br>Arvo Koistinen<br>Esa Leppänen<br>Anna-Mari Koski | Kuusamo health care center                          | Erja Isopoussu<br>Tapio Kääriäinen<br>Eero Vierimaa<br>Esa Kilkki<br>Liisa Riihelä<br>Mare Riihelä<br>Leena Ryysy<br>Reino Paldanius<br>Taina Meriläinen<br>Paula Poukka<br>Tuula Tikkanen<br>Riitta Savolainen |
| Kirkkonummi health care center   | Anja Lappalainen<br>Maisa Liimatainen<br>Jaana Santaholma                                                                                                                         | Kuusankoski Hospital                                | Esa Kilkki<br>Liisa Riihelä<br>Mare Riihelä<br>Leena Ryysy<br>Reino Paldanius<br>Taina Meriläinen<br>Paula Poukka<br>Tuula Tikkanen<br>Riitta Savolainen                                                        |
| Kivelä Hospital, Helsinki        | Ari Aimolahti<br>Maarit Kärki                                                                                                                                                     | Kymenlaakso Central Hospital                        | Aulikki Mäkelä<br>Marjukka Tanner<br>Eija Behm                                                                                                                                                                  |
| Koskela Hospital, Helsinki       | Maija Lehtimäki<br>Nina Uhlenius<br>Eeva Huovinen<br>Veikko Ilkka                                                                                                                 | Laakso Hospital, Helsinki                           | Pirkko Nyländen<br>Hilkka Laukkanen<br>Pirjo Myllykoski<br>Inge-Maj Jousmaa<br>Jukka Rinne                                                                                                                      |
| Kotka health care center         | Eeva Pälikkö-Kontinen<br>Annika Vanhanen                                                                                                                                          | Lahti City Hospital                                 | Pirjo Linkola<br>Irja Pulli                                                                                                                                                                                     |
| Kouvola health care center       | Erkki Koskinen<br>Anna Borgman<br>Taimi Siitonen                                                                                                                                  | Länsi-Pohja Central Hospital                        | Sirkka Severinkangas<br>Timo Tulokas                                                                                                                                                                            |
|                                  |                                                                                                                                                                                   | Länsi-Uusimaa Hospital                              |                                                                                                                                                                                                                 |
|                                  |                                                                                                                                                                                   | Lappeenranta health care center,<br>Armila Hospital |                                                                                                                                                                                                                 |
|                                  |                                                                                                                                                                                   | Lappi Central Hospital                              |                                                                                                                                                                                                                 |



|                               |                                                                                                                               |                                                                               |                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lohja Hospital                | Liisa Hyvärinen<br>Tuula Granlund<br>Mikko Saari<br>Tuula Salonen                                                             | Palokka health care center, Palokka<br>Palokka health care center, Vaajakoski | Tarja Tick-Sinkkilä<br>Leena Welling<br>Kirsti Mäkinen<br>Päivi Sopanen                                                                                                                                                                                                                                                                                                |
| Loimaa health care center     | Reija Tähtinen<br>Pirjo Eloranta<br>Arja Mäkelä                                                                               | Pieksämäki Hospital                                                           | Mari Tamminen<br>Markku Taskinen<br>Maj-Len Holmbäck                                                                                                                                                                                                                                                                                                                   |
| Lounais-Häme Hospital         | Tarja Kalliomäki<br>Juha Koskelainen<br>Riitta Nikkanen<br>Vesa Salonen<br>Niilo Savolainen<br>Hannu Sulonen<br>Eija Valtonen | Pietarsaari Hospital                                                          | Bo Isomaa<br>Leena Sarelin<br>Laura Hyttinen<br>Päivi Kekäläinen<br>Maija Pietarinen<br>Anneli Rissanen<br>Risto Laitinen<br>Hannu Turtola<br>Ulla Maija Henttula                                                                                                                                                                                                      |
| Malmi Hospital, Helsinki      | Helena Lanki<br>Seija Moilanen<br>Marju Tilly-Kiesi                                                                           | Pori City Hospital                                                            | Pirjo Merensalo<br>Kirsi Sävelä<br>Terttu Viitala<br>Päivi Ahonen<br>Heidi Johansson<br>Sirikka Rämö<br>Bjarne Rask<br>Marjatta Kallion<br>Anne Holma<br>Matti Honkala<br>Aino Tuomivaara<br>Riitta Vainionpää<br>Kaisa Laine<br>Taru Salminen<br>Paula Ketola<br>Kaisa Saarinen<br>Pirkko Aalto<br>Marja Lanni<br>Irmeli Laitaharju<br>Eija Immonen<br>Leena Juurinen |
| Mänttä Hospital               | Anne-Mirjami Hänninen<br>Ismo Pirttiniemi                                                                                     |                                                                               |                                                                                                                                                                                                                                                                                                                                                                        |
| Mikkeli Central Hospital      | Anita Gynther<br>Maarit Salminen<br>Tuomo Vänttinen                                                                           | Porvoo Hospital                                                               |                                                                                                                                                                                                                                                                                                                                                                        |
| Nurmijärvi health care center | Mikko Honkasalo<br>Marko Miettinen<br>Päivi Nurminen                                                                          | Raahe Hospital                                                                |                                                                                                                                                                                                                                                                                                                                                                        |
| Oulaskangas Hospital          | Elina Jokelainen<br>Pirkko-Liisa Jylkkä<br>Elsi Kaarlela<br>Jari Vuolaspuro                                                   | Rauma Hospital                                                                |                                                                                                                                                                                                                                                                                                                                                                        |
| Oulu health care center       | Rauni Häkkinen<br>Liisa Hiltunen<br>Sirikka Keinänen-Kiukaanniemi                                                             |                                                                               |                                                                                                                                                                                                                                                                                                                                                                        |
| Päijät-Häme Central Hospital  | Hannu Haapamäki<br>Satu Hämäläinen<br>Antero Helanterä<br>Vesa Ilvesmäki<br>Hilkka Miettinen                                  | Riihimäki Hospital                                                            |                                                                                                                                                                                                                                                                                                                                                                        |

|                                     |                                                                                                                                                                                                                  |                                                                                         |                                                                                                                                                                                                                               |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Salo Hospital                       | Jouni Lapinleimu<br>Pirkko Rautio<br>Merja Virtanen<br>Anita Alanko                                                                                                                                              |                                                                                         | Heikki Oksala<br>Tuija Oksanen<br>Kristiina Salonen<br>Sirkku Tulokas                                                                                                                                                         |
| Satakunta Central Hospital          | Markku Asola<br>Paavo Pääkkönen<br>Marjo Pentti<br>Marja Rautavirta<br>Pertti Kunelius<br>Sirkku Mannila                                                                                                         | Tiirismaa health care center<br><br>Turku health care center                            | Hilkka Tauriainen<br>Leena Petlin<br>Liisa Savolainen<br>Tiina Kivelä<br>Anne Artukka<br>Irmeli Hämäläinen                                                                                                                    |
| Savonlinna Central Hospital         | Tuula Pulli<br>Päivi Sallinen<br>Eija Toivanen<br>Helena Valtonen<br>Aarne Vartia                                                                                                                                |                                                                                         | Päivi Kokkila<br>Lea Sorvari<br>Markku Vähätalo<br>Hille Virtamo                                                                                                                                                              |
| Seinäjoki Central Hospital          | Eeva Korpi-Hyövälti<br>Terhikki Latvala<br>Esko Leijala<br>Tarja Nyrhilä                                                                                                                                         | Turku University Central Hospital                                                       | Kaija Breitholz<br>Raija Eskola<br>Kaj Metsärinne<br>Riikka Neva<br>Ulla Pietilä                                                                                                                                              |
| Tammisaari Hospital                 | Jukka Rinne<br>Inge-Maj Jousma                                                                                                                                                                                   |                                                                                         | Pirjo Saarinen<br>Riitta Tuominen                                                                                                                                                                                             |
| Tampere health care center          | Pekka Alarotu<br>Liisa Calonius<br>Seppo Gummerus<br>Paula Helin<br>Tarja Kaitala<br>Helena Kirkkopelto-Jokinen<br>Eila Kujansuu<br>Taru Niskanen<br>Atte Vadén<br>Timo Saaristo<br>Marjo Kuortti<br>Arja Kallio | Vaajakoski health care center<br><br>Vaasa Central Hospital<br><br>Valkeakoski Hospital | Markku Asola<br>Sari Äyräpää<br>Päivi Sopanen<br>Kirsti Mäkinen<br>Stig Bergkulla<br>Ulla Hautamäki<br>Vivi-Ann Myllyniemi<br>Irma Rusk<br>Terhi Immonen<br>Seppo Ojanen<br>Maisa Rautiainen<br>Eija Valtonen<br>Hannu Ylönen |
| Tampere University Central Hospital | Ilpo Ala-Houhala<br>Tuula Kuningas<br>Paula Lampinen<br>Marita Määttä                                                                                                                                            | Vammala Hospital                                                                        | Irma Isomäki<br>Leena Mustaniemi<br>Merja Tapiolinna-Mäkela                                                                                                                                                                   |

|                                         |                                                                                                             |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------|
| Vantaa health care center, Korso        | Riitta Kroneld<br>Jaana Vahtola                                                                             |
| Vantaa health care center, Länsimäki    | Riitta Toivonen<br>Raila Ahonen<br>Marja Ivaska-Suomela<br>Anu Jauhiainen<br>Sari Kultti<br>Pirjo Kiviniemi |
| Vantaa health care center, Martinlaakso | Merja Laine<br>Tiina Pellonpää<br>Riitta Puranen                                                            |
| Vantaa health care center, Myyrmäki     | Anne Airas<br>Jukka Laakso                                                                                  |
| Vantaa health care center, Rekola       | Matti Eerola<br>Eeva Jatkola                                                                                |
| Vantaa health care center, Tikkurila    | Riitta Lönnblad<br>Jari Mäkelä<br>Anita Malm<br>Elli Rautamo                                                |
| Åland Central Hospital                  | Hans Granlund<br>Ann-Christin Johnsson<br>Monica Forsen                                                     |
| Äänekoski health care center            | Satu Savolainen<br>Tapani Marjanen                                                                          |