Precision Medicine Programmes at Ludger

Glycomics, Diabetes and Fat Bellies

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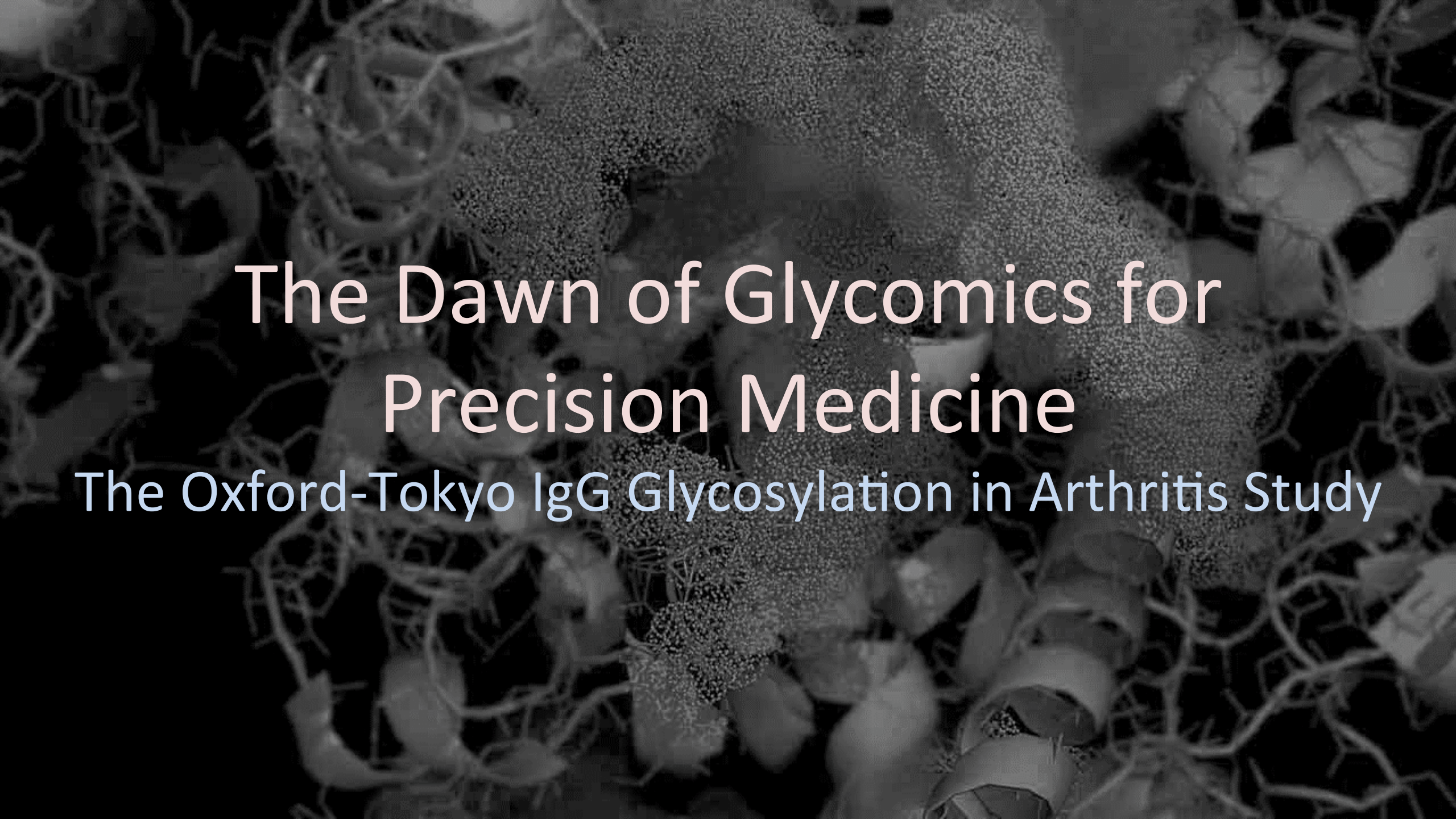
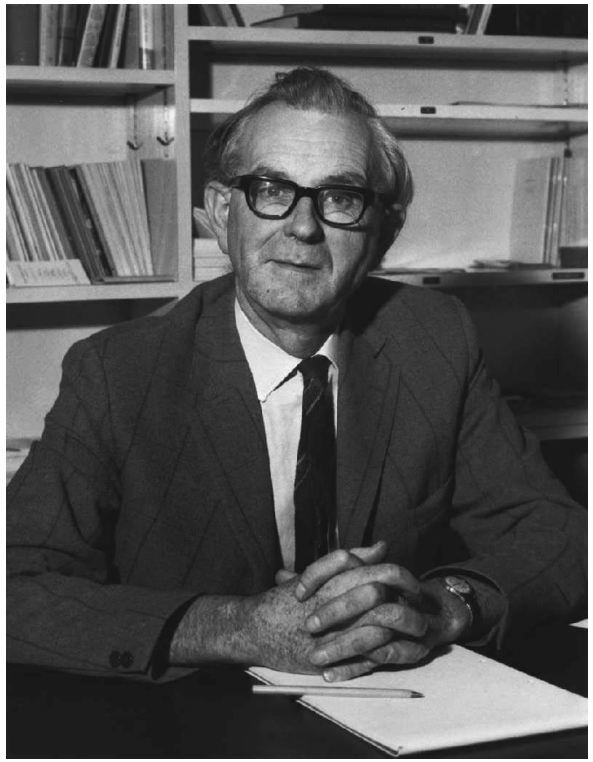
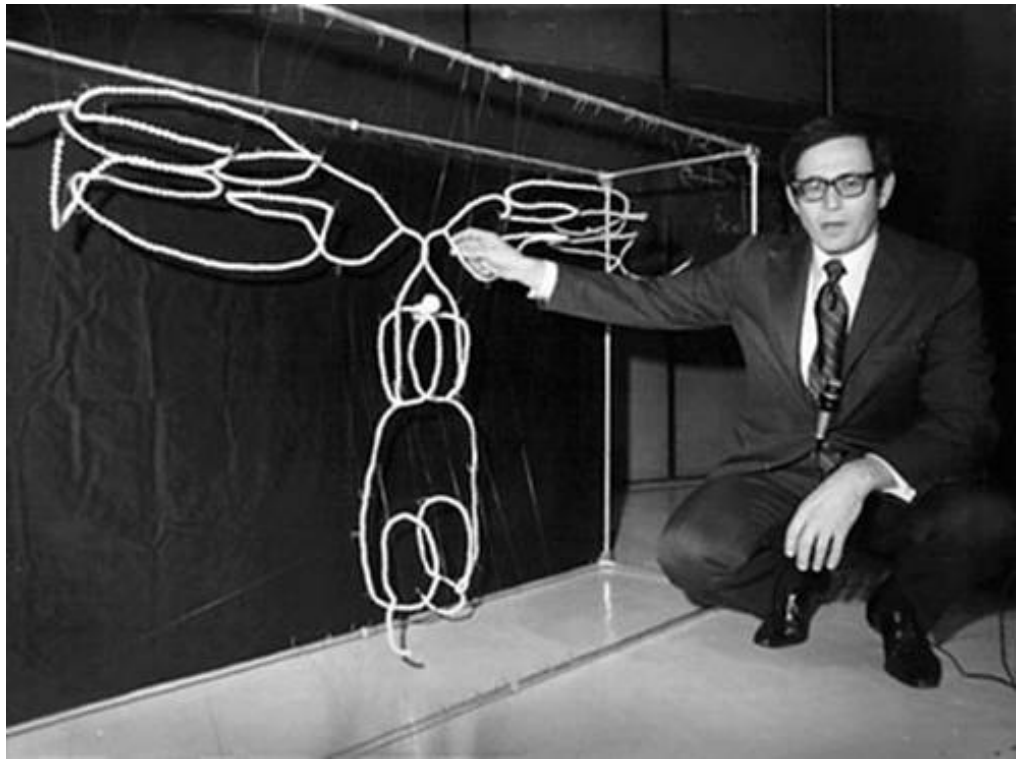


The Dawn of Glycomics for Precision Medicine

The Oxford-Tokyo IgG Glycosylation in Arthritis Study

Profs Porter and Edelman: 1972 Nobel Laureates for elucidation of IgG structure





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Whitley Professor of Biochemistry, University of Oxford

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'Pioneer of the glycobiology of N-linked sugar chains'

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Structures of the Asparagine-linked Sugar Chains of Subcomponent C1q of the First Component of Bovine Complement*

(Received for publication, June 18, 1982)

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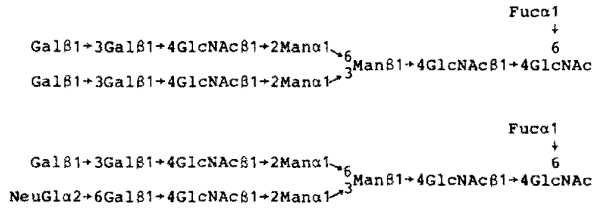
Bovine C1q, a subcomponent of the first component of complement, contains six asparagine-linked sugar chains in 1 molecule. The sugar chains are exclusively distributed in the noncollagenous regions.

The sugar chains were liberated as radioactive oligosaccharides from the polypeptide portion by hydrazinolysis followed by *N*-acetylation and NaB[^{3}H]$_4$ reduction, and their structures were studied by sequential exoglycosidase digestion in combination with methylation analysis. Bovine C1q was shown to contain equal amounts of neutral and acidic oligosaccharides with the following structures:



where NeuGl is *N*-glycolylneuraminic acid.

C1q was purified to homogeneity (4). It was found that bovine C1q has a molecular structure similar to that of human C1q and can be substituted for human C1q in reconstructing active C1 with purified human C1r and C1s (4). However, bovine C1q, in contrast to human C1q, could not aggregate latex particles coated with human IgG (4). Because the carbohydrate composition of bovine C1q was slightly different from that of human C1q although the amino acid composition was very similar (4), structural differences in the sugar chain moieties of both C1q samples could be a cause of the difference in their Fc-binding activities. This paper will describe structural studies on the asparagine-linked sugar chains of bovine C1q and their location in the glycoprotein molecule.

EXPERIMENTAL PROCEDURES[2]

RESULTS

Liberation of the Sugar Chains of Bovine C1q as Oligosaccharides— In order to determine the number of asparagine-linked sugar chains in 1 molecule, a time course study of the liberation of oligosaccharide by hydrazinolysis was performed by using 1 mg of bovine C1q and 6′-sialyllactose as an internal standard as described in detail previously (9). Based on the radioactivities of tritium incorporated into 6′-sialyllac-

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IgG Glycosylation Changes in RA and OA Patients

Parekh et al (1985) Nature 316:452-457

Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG

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Rheumatoid arthritis (RA) is a widely prevalent (1–3%) chronic systemic disease thought to have an autoimmune component[1]; both humoral[1–4] and cellular[5,6] mechanisms have been implicated. Primary osteoarthritis (OA) is considered to be distinct from rheumatoid arthritis, and here damage is thought to be secondary to cartilage degeneration. In rheumatoid arthritis, immune complexes are present that consist exclusively of immunoglobulin[7], implying that this is both the 'antibody' (rheumatoid factor [RF]) and the 'antigen' (most commonly IgG). Autoantigenic reactivity has been localized to the constant-region ($C_\gamma 2$) domains of IgG[8,9]. There is no evidence for a polypeptide determinant but carbohydrate changes have been reported[10]. We have therefore conducted a study, simultaneously in Oxford and Tokyo, to compare in detail the *N*-glycosylation pattern of serum IgG (Fig. 1) isolated from normal individuals and from patients with either primary osteoarthritis or rheumatoid arthritis. The results, which required an evaluation of the primary sequences of ~1,400 oligosaccharides from 46 IgG samples, indicate that: (1) IgG isolated from normal individuals, patients with RA and patients with OA contains different distributions of asparagine-linked bi-antennary complex-type oligosaccharide structures, (2) in neither disease is the IgG associated with novel oligosaccharide structures, but the observed differences are due to changes in the relative extent of galactosylation compared with normal individuals. This change results in a 'shift' in the population of IgG molecules towards those carrying complex oligosaccharides, one or both of whose arms terminate in *N*-acetylglucosamine. These two arthritides may therefore be glycosylation diseases, reflecting changes in the intracellular processing, or post-secretory degradation of *N*-linked oligosaccharides.

At least 30 different complex-type bi-antennary oligosaccharides are associated with human serum IgG (Fig. 2). To compare the molar proportions of each of these structures, each serum IgG sample was subjected to controlled hydrazinolysis to release intact its associated oligosaccharide moieties[11]. Reduction of the reducing terminal *N*-acetylglucosamine residues using NaB^3H_4 was then performed to label radioactively each carbohydrate chain. Each labelled oligosaccharide mixture was subjected to exhaustive neuraminidase digestion in order to analyse the distribution of neutral structures. The resulting 'asialo' oligosaccharide mixtures were fractionated by Bio-Gel P-4 (~400 mesh) gel filtration chromatography, a technique that separates neutral oligosaccharides on the basis of their effective hydrodynamic volumes[12] (Figs 2, 3).

A comparison of the individual profiles obtained reveals several interesting points. First, all P-4 chromatograms of asialo mixtures from control individuals (Fig. 3) were essentially identical to one another (data not shown) and similar to those reported previously[13]. As any individual IgG molecule contains, on average, 2.8 *N*-linked oligosaccharides[14], many different IgG subpopulations must exist, each unique with respect to the sequence of its associated oligosaccharides. Further, the overall relative molar contribution of each of the 30 or so structures in the analysis of polyclonal IgG is remarkably constant. The occurrence of this large number of different oligosaccharides is not the result of performing the analysis on polyclonal IgG, because this same 'set' of structures is found on human myeloma proteins[13] and an analogous situation exists for mouse monoclonal antibodies[14]. Second, the P-4 chromatograms of asialo mixtures from patients with rheumatoid arthritis are also essentially the same between patients (data not shown), but differ from control profiles (Fig. 3). Third, the asialo oligosaccharide

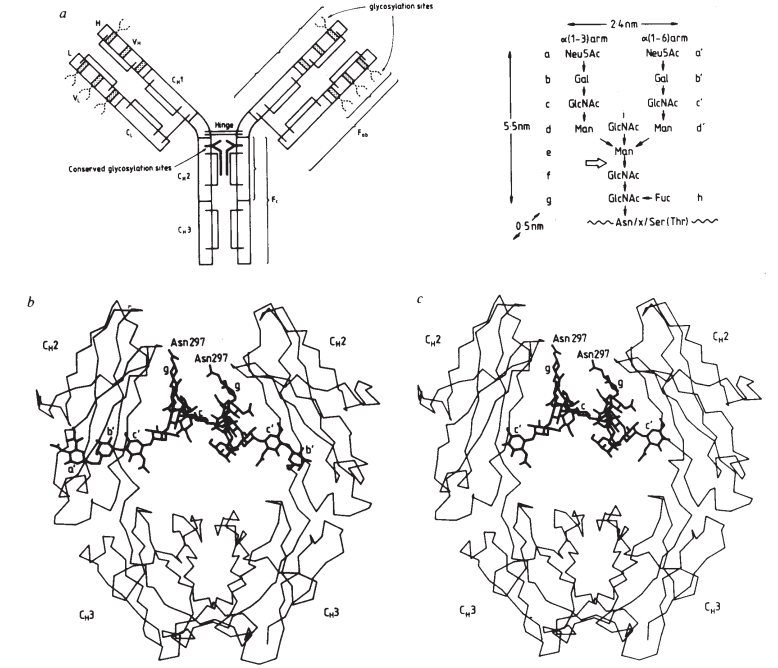


Fig. 1 **a**, The antibody molecule consists of two heavy (H) and two light (L) chains, linked by disulphide bridges (solid lines) and is divided into homologous regions of sequence (V_H, C_H1, C_H2 and C_H3), each of which has an intra-chain disulphide bridge. (The pattern of inter-chain disulphide bridging shown here is characteristic of human subclass IgG1.) In V_H and V_L, the dotted segments represent the hypervariable regions of sequence that, in the three-dimensional structure, together form the antigen-binding site. The conserved asparagine-linked bi-antennary complex oligosaccharide chains are attached to Asn 297 in the C_H2 domains[16]. Oligosaccharide attachment sites are found in the Fab region. Their frequency and location are dependent on the presence of Asn-X-Ser sites in the hypervariable regions[27,28]. The relative size of an immunoglobulin domain and a fully extended *N*-linked complex oligosaccharide[29] are similar. Complex-type oligosaccharides present on IgG can be subdivided into an outer-arm region (a, a′, b, b′, c, c′), and the core, which is composed of a trimannosyl unit (d, d′, e) and a *N*,*N*′-diacetylchitobiosyl unit (f, g). The 'bisect' GlcNAc (residue i) is linked β(1-4) and the fucose (residue h) is linked α(1-6). The arrow between residues e and f indicates the site of interaction between the two oligosaccharides in *b* and *c*. **b**, Refined structure at 2.8 Å of rabbit Fc fragment from the crystal data of Sutton and Phillips (ref. 16 and B. J. Sutton, unpublished data). The two carbohydrate chains, each attached at Asn 297, differ in conformation and may also differ in sequence and bridge the two C_H2 domains. The α(1-3) arm of the chain (left side) is always devoid of galactose and interacts through its β(1-2)-linked GlcNAc residue (c) with the Manβ(1-4) GlcNAc segment of the opposing (right side) oligosaccharide chain (see *a*). The α(1-3) arm of the right chain extends outwards between the domains with no apparent steric constraints on its length. A Neu5Ac unit (a′) is shown on one α(1-6) arm only (left). The electron density for this unit is weak, and experimentally, no disialylated oligosaccharide chains occur on the Fc[14]. The extent of oligosaccharide heterogeneity in a single crystal is identical to that found in pooled Fc fragments[14,30], consequently the X-ray data represent the composite structure. **c**, Fc fragment containing oligosaccharides devoid of galactose and sialic acid on each of the (α1→6) arms. Since these residues in normal IgG are in contact with the surface of the protein (see *b*), their absence vacates oligosaccharide-binding sites in IgG from arthritic patients and could make the IgG 'sticky' by creating a lectin-like activity. It is not known to what extent the remaining sugar residues remain in contact with the peptide.

P-4 chromatograms of osteoarthritic (OA) patients are also characteristic of all such patients and are distinct from both the control and rheumatoid arthritic (RA) profiles (Fig. 3). Finally, the differences between the control and arthritic (both osteoarthritic and rheumatoid) P-4 profiles can be rationalized in terms of a population shift towards oligosaccharide structures of lower hydrodynamic volume. To establish the molecular basis of this shift, the asialo oligosaccharides from each patient were analysed with respect to their relative levels of different core substitutions and the degree and nature of their outer-arm substitutions (see Figs 1, 4).

For the asparagine-linked oligosaccharides of IgG, core substitutions can be readily determined by digesting the pool of oligosaccharides with a mixture of *Streptococcus pneumoniae*

> 5 Person-Years for Detailed Glycoanalysis of 46 IgG Samples

HT glycoprofiling in 1984: 1,400 samples analysed

Table 1 Per cent oligosaccharide chains lacking galactose in serum IgG

Individual patients	Control		Osteoarthritis		Rheumatoid arthritis		Duration disease (yr)	Serology
Oxford series	Oxf1	26.7	Oxf7f*	38.9	Oxf13f	51.5	25	+
	Oxf2	23.9	Oxf8f*	36.0	Oxf14f	53.7	18	+
	Oxf3	26.2	Oxf9f	26.5	Oxf15f	41.9	2	+
	Oxf4	23.4	Oxf10m	35.2	Oxf16f	43.6	13	−
	Oxf5	16.5	Oxf11f	31.4	Oxf17f	54.9	3	+
	Oxf6	19.3	Oxf12f	33.0	Oxf18f	55.0	23	−
Tokyo series	Tok1f	31.4	Tok9f	32.2	Tok16m	44.3	4	+
	Tok2m	26.8	Tok10f	32.7	Tok17f	53.8	10	+
	Tok3m	38.9	Tok11f	32.7	Tok18f	52.4	16	+
	Tok4m	25.4	Tok12f*	47.4	Tok19f	48.4	13	+
	Tok5f	23.3	Tok13f	43.2	Tok20m	43.9	16	+
	Tok6f	19.4	Tok14f	33.4	Tok21f	47.9	18	+
	Tok7m	28.7	Tok15f	35.4				
	Tok8f	25.7						
Mixed series					O/T1m	74.5	18	+
					O/T2f	56.2	27	+
					O/T3m	55.6	>15	+
					O/T4f	51.8	15	+
					O/T5m	44.3	15	+
					O/T6m	38.7	>10	+
Mean ± s.d.	Oxf	22.7 ± 4.0		33.5 ± 4.3		50.1 ± 5.9		
	Tok	27.5 ± 5.8		36.7 ± 6.1		51.0 ± 9.1		
Pooled control serum	Bern-1†	24.0		—		—		

Non-galactosylated oligosaccharides were isolated as follows. Asialo oligosaccharides (1×10^7 c.p.m.) from the IgG of each individual were applied to an RCA-120 agarose column (Miles-Yeda, Lot no. AR26) of dimensions 0.6 × 20 cm. The column was developed in 5 mM sodium acetate (*p*H 5.6). Non-galactosylated structures eluted in the void while digalactosylated and monogalactosylated structures eluted later and at unique volumes (data not shown). The number of galactose residues in each peak was confirmed by following the change in hydrodynamic volume after digestion with jack bean β-*N*-acetyl hexosaminidase (14 μM substrate, 150 U ml^{-1} of enzyme in 0.1 M citrate phosphate, *p*H 4.5). For the Oxford series, the differences in the means were significant, with $P = 0.002$ (C vs OA) and $P < 0.001$ (C vs RA) and $P = 0.002$ (OA vs RA). For the Tokyo series (including data from Tok and O/T patients), the significance was $P = 0.006$ (C vs OA), $P < 0.001$ (C vs RA) and $P = 0.0008$ (OA vs RA). Any difference in the means between the two series was not considered statistically significant and no correlation was found between the extent of galactosylation and age and sex. The ratios of digalactosylated to monogalactosylated structures obtained as described in the text were as follows: C, 0.89 ± 0.09; OA, 0.75 ± 0.15; RA, 0.63 ± 0.12. The statistical significance of these is $P = 0.07$ (C vs OA), $P = 0.0003$ (C vs RA), $P > 0.1$ (OA vs RA). Statistical analysis was performed using a non-parametric combined-order statistic test (Wilcoxon–Mann–Whitney test[25]). Probabilities are quoted for a two-tailed test with a null hypothesis H_0: $\mu_1 = \mu_2$ tested against an alternative hypothesis H_1: $\mu_1 \neq \mu_2$. The mixed series refers to serum samples from British individuals, where the IgG was purified in England and sugar analysis performed in Tokyo. Blood samples in the Oxford series were obtained from patients at St John's Highfield Hospital, Droitwich, UK, and the Queen Elizabeth Medical Centre, Birmingham, UK. Patients Oxf13 to Oxf18, Tok16 to Tok21 and O/T1 to O/T6 had active disease and all fulfilled the American Rheumatism Association criteria for definite or classical rheumatoid arthritis[26]. The Oxf patients range in age was 50–75 yr (mean, RA 64 ± 8 s.d.; OA, 68 ± 9 s.d.), and the Tok patients 41–75 yr (mean, RA 57 ± 14 s.d.; OA, 58 ± 9 s.d.). Control serum in the Oxf series was obtained from random blood bank donors. The Tok series control serum was from donors, age range 22–47 yr (mean 38 ± 9 s.d.). The analysis was performed double-blind, with clinical histories obtained after completion of oligosaccharide analysis.

* Patients Oxf7 and Oxf8 had long-standing osteoarthritis and very recently have been showing signs of an inflammatory component (6 months and 24 months respectively). The patient Tok 12 (aged 74 yr) was originally diagnosed as osteoarthritic but now also has Sjögren's syndrome.

† Bern-1 refers to IgG from the pooled serum of several thousand individuals and was given by Dr U. Nydegger of the Blood Transfusion Centre of the Swiss Red Cross, Bern, Switzerland.

β-galactosidase and β-*N*-acetylhexosaminidase[13]. The resulting digestion products are diagnostic for each of the four cores and differ sufficiently in hydrodynamic volume to be resolved on a P-4 column. The results (Fig. 4) indicate clearly that there is no systematic correlation between disease state and the incidence of any particular type of core structure (that is, no apparent changes in the levels of the enzymes GnT III (ref. 15) and $\alpha(1 \rightarrow 6)$fucosyltransferase).

The outer-arm structures can be characterized with respect to the incidence, linkage and location of galactose, *N*-acetylglucosamine and *N*-acetylneuraminic acid. The asialo oligosaccharide mixtures were therefore subjected to *Ricinus communis* lectin–agarose affinity chromatography to separate galactosylated and non-galactosylated structures. Table 1 shows that in control IgG, ~75% of all oligosaccharide chains contain at least one galactose residue. In IgG isolated from rheumatoid and osteoarthritic patients, only ~50% ($P = 0.001$) and ~65% ($P = 0.002$), respectively, of the chains contained galactose. The ratio in each individual case of digalactosyl to monogalactosyl structures was determined either by chromatography of the galactosylated oligosaccharides on *R. communis* lectin–agarose, or enzymatically. In the latter, digestion with jack bean β-*N*-acetylhexosaminidase, followed by P-4 gel permeation chromatography, results in the resolution of fragments diagnostic of the digalactosylated and monogalactosylated structures. The ratios of the digalactosyl to monogalactosyl structures obtained by both methods were consistent and indicated respective decreases of 30% ($P = 0.0003$) and 15% ($P = 0.07$) in the rheumatoid and osteoarthritic asialo oligosaccharide mixtures.

To determine whether the decreased number of chains containing galactose was secondary to a decrease in outer-arm $\beta(1\text{–}2)$ linked *N*-acetylglucosamine residues, the asialo oligosaccharide structures from each individual were digested with jack bean β-galactosidase and the resulting degalactosylated oligosaccharides subjected to P-4 chromatography. In all three groups essentially identical profiles were found, implying no deficiency in outer-arm $\beta(1\text{–}2)$-linked *N*-acetylglucosamine residues (GlcNAc 5 and 5′, Fig. 2). There can, therefore, be no difference between individuals, regardless of their disease state, with respect to the extent of *N*-acetylglucosaminylation (that is, no apparent change in the levels of enzymes GnT I and GnT II; (ref. 15)).

As the negatively charged *N*-acetylneuraminic acid residues confer mobility in an electric field, an aliquot of the labelled

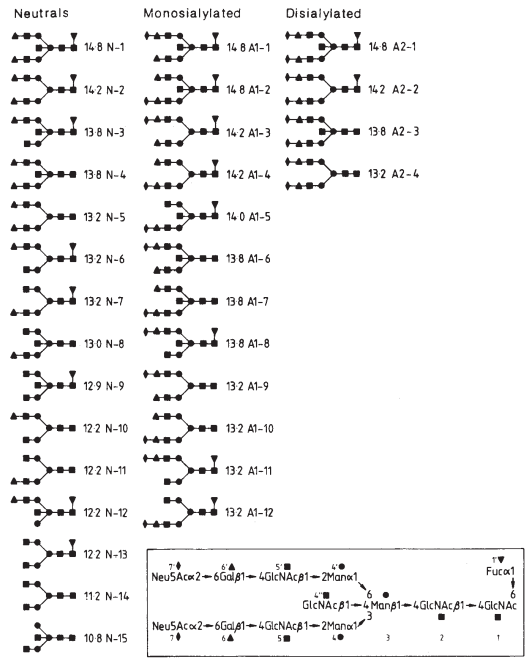


Fig. 2 Primary sequences of the *N*-linked oligosaccharides associated with human IgG. The hydrodynamic volume (as measured in glucose units) of each structure (or of its neutral derivative in the case of those sialylated) is indicated (refs 12, 13 and R.B.P. *et al.*, unpublished data), and was determined by comparison with $\alpha(1\text{–}6)$-linked glucose oligomer standards.

oligosaccharide pool (before neuraminidase digestion) was subjected to high-voltage paper electrophoresis. The oligosaccharides were completely separated into neutral, monosialylated and disialylated structures. The occurrence of these structures is represented in Fig. 4 and the structures present in the three peaks are detailed in Fig. 2. Within each study the osteoarthritis and rheumatoid IgGs show a slight decrease in the number of chains terminating with one or two *N*-acetylneuraminic acid residues, consistent with their decrease in galactosylation.

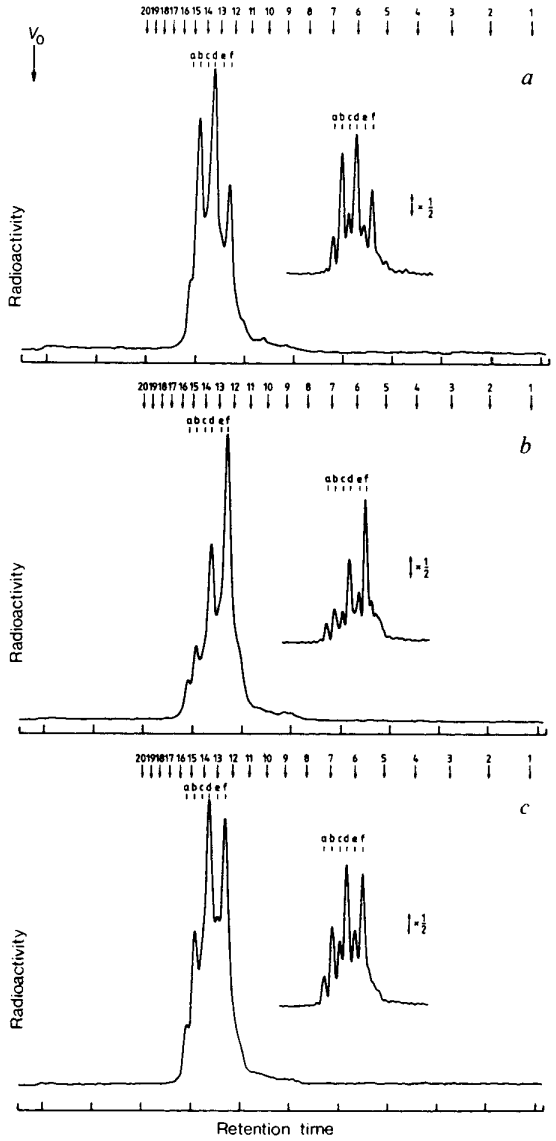
The results show that osteoarthritis, and particularly rheumatoid arthritis, are associated with marked changes in the level of outer-arm galactosylation of the complex *N*-linked oligosaccharides of serum IgG ($P = 0.002$ OA versus control (C), $P = 0.001$ RA versus C) and these also distinguish the disease states ($P = 0.002$ OA versus RA). Importantly, no novel oligosaccharides were found on IgG from either of the arthritides (data not shown).

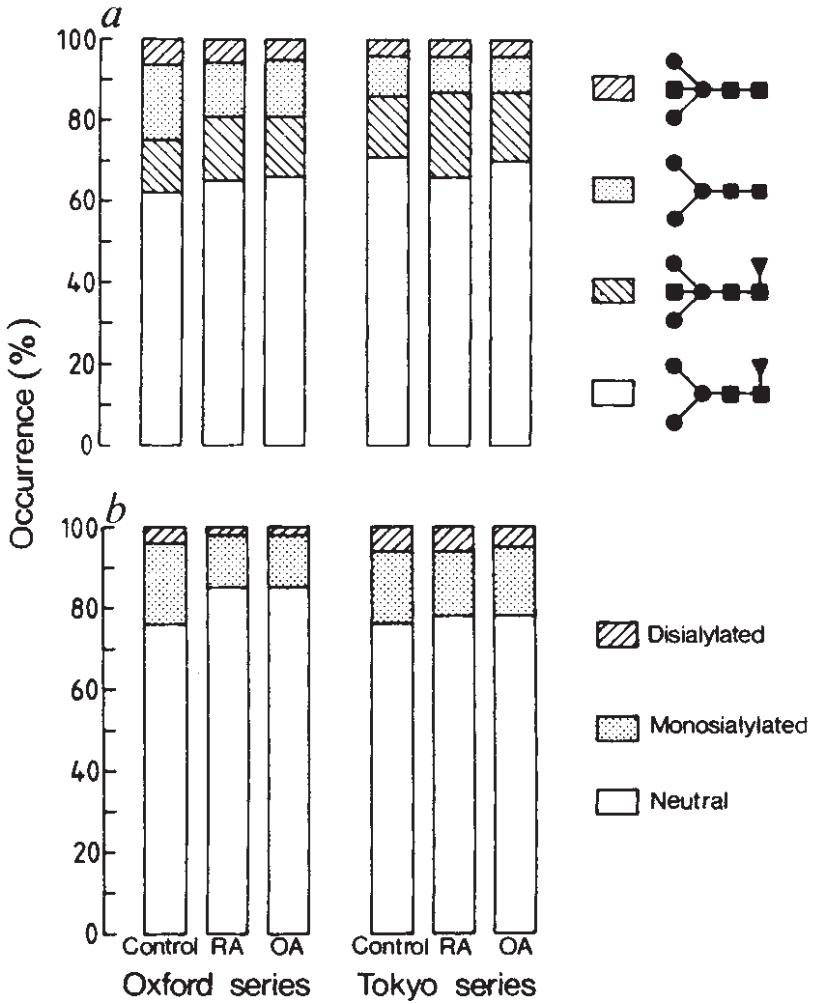
A possible consequence of this change in galactosylation is suggested by recent X-ray crystallographic studies on rabbit Fc, which have helped to define in molecular terms the manner in which the opposing oligosaccharides interact with each other and with the peptide[16]. In particular, the oligosaccharide chains appear to have different primary sequences (and hence many IgG molecules are structurally asymmetrical) and conformations (Fig. 1*b*). The $\alpha(1\text{–}6)$ arms interact with the protein surface, the major contacts being through their Neu5Ac$\alpha(2\text{–}6)$Gal$\beta(1\text{–}4)$GlcNAc segment. The rigid[17] $\alpha(1\text{–}3)$ arm of at least one of the paired oligosaccharides is always devoid of galactose, thereby exposing its outer-arm $\beta(1 \rightarrow 2)$ linked *N*-acetylglucosamine residue, which then interacts directly with the Man$\beta(1\text{–}4)$GlcNAc segment of the opposing carbohydrate chain. The $\alpha(1\text{–}3)$ arm of the latter oligosaccharide extends outwards between the domains with no apparent steric constraints on its length (Fig. 1*b*).

Given this minimum restriction on pairing of oligosaccharides in Fc, a probability analysis reveals that the above changes in galactosylation would lead to a marked elevation in the incidence of Fc molecules that totally lack galactose (~300% in rheumatoid arthritis and ~90% in osteoarthritis, that is, from 10% in normal serum to 19% in osteoarthritis and 40% in rheumatoid arthritis). Thus, the hierarchical set of changes, beginning with an altered level of galactosylation and proceeding via a change in the relative populations of a constant set of oligosaccharide structures, leads through the phenomenon of pairing in the Fc, to dramatic changes in the incidence of individual Fc subpopulations. Such a change in IgG could result in the formation and/or persistence of immunoglobulin complexes by any one of several possible mechanisms that may or may not involve a true autoimmune response. First, oligosaccharides now terminating in *N*-acetylglucosamine could expose previously masked protein determinants or create new protein–oligosaccharide determinants that may be immunogenic. Second, an increase in the level of certain IgG subpopulations could lead to the exposure of certain Fc determinants at much higher concentrations than before. This could elicit a new immune response or raise a pre-existing low-level (subclinical)

IgG Glycosylation Patterns Can Be Used To Stratify RA and OA Patients

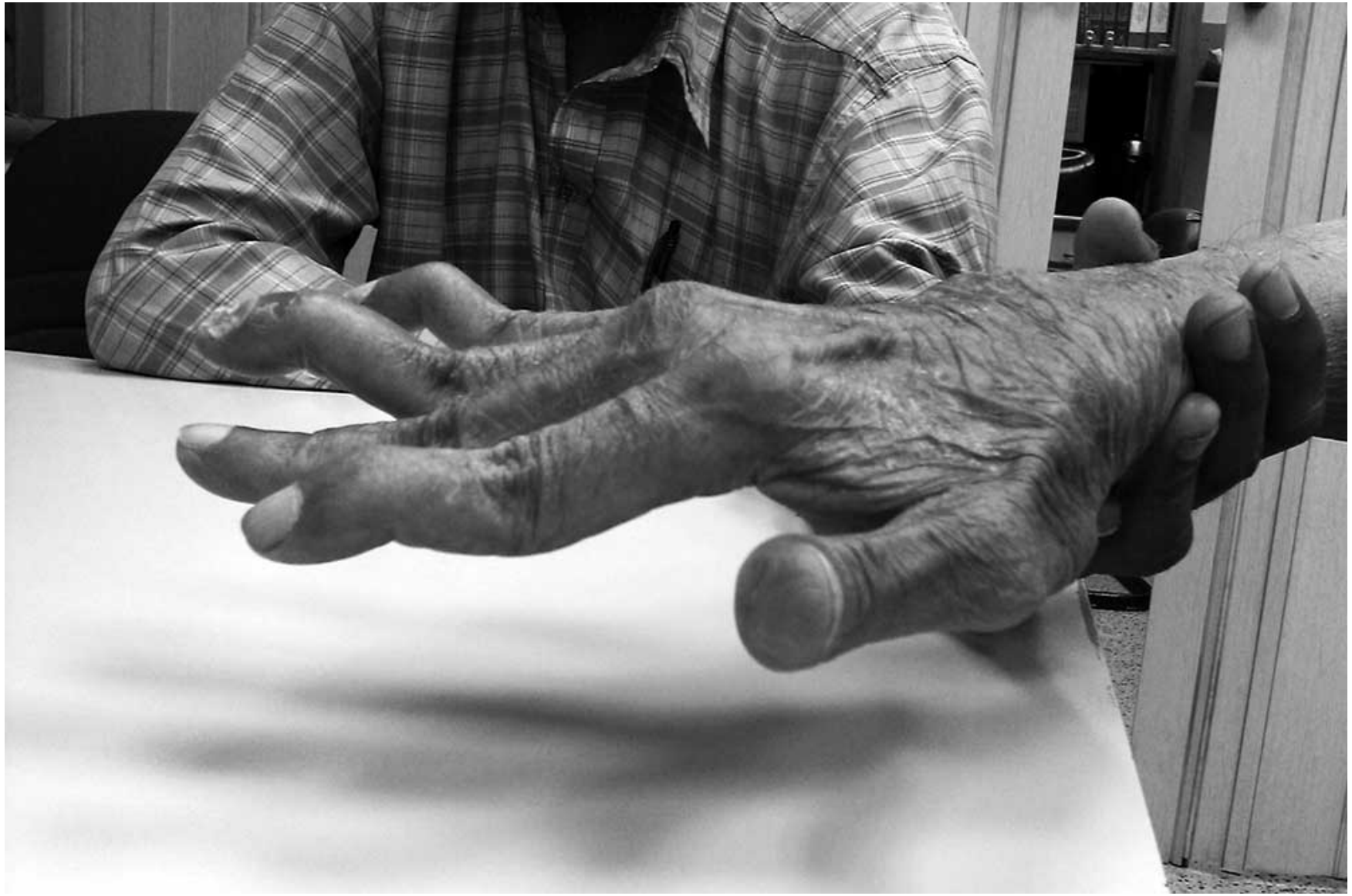
For a given population, changes from 'normal' patterns indicate disease





The Hope: Tracking IgG Glycosylation Could Help Patients

But how? Easier said than done




Glycosylation and Disease:
30 Years On ...

Expanded Studies on IgG Glycosylation and RA

Galactosylation is strongly associated with disease state

Journal of proteome research

Article

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Association between Galactosylation of Immunoglobulin G and Improvement of Rheumatoid Arthritis during Pregnancy Is Independent of Sialylation

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Supporting Information

ABSTRACT: Rheumatoid arthritis (RA) is known to improve during pregnancy and to flare after delivery. Changes in the glycosylation of immunoglobulin G (IgG)'s fragment crystallizable (Fc) have been suggested to play a role herein. Recent animal studies indicate that not galactosylation but mainly sialylation is important in this respect. We aim to find new associations between IgG-Fc N-glycosylation and improvement of RA during pregnancy and the flare after delivery. Sera of RA patients (n = 251 pregnancies) and healthy controls (n = 32), all participating in a prospective cohort study on RA and pregnancy (PARA study), were collected before conception, during pregnancy, and after delivery. Using a recently developed fast and robust nanoRP-HPLC-sheath-flow-ESI−MS method the glycosylation of IgG Fc-glycopeptides was measured in a subclass specific manner, with relative standard deviations of <4% for the 8 most abundant IgG Fc glycopeptides during the entire measurement period of over 3 weeks. In patients and controls, several glycosylation changes were observed during pregnancy. In depth analysis of the association of these glycosylation changes with disease activity revealed that galactosylation, independent of sialylation, is associated with improvement of RA during pregnancy. Functional studies in human cell systems should be performed to obtain more insight into this matter.

KEYWORDS: *rheumatoid arthritis, pregnancy, glycosylation, immunoglobulin G, galactosylation, sialylation, fucosylation, bisecting GlcNAc*

INTRODUCTION

Autoimmune diseases provide a large economic threat in industrialized countries. Rheumatoid arthritis (RA) is one of these diseases, affecting 0.5−1% of the adult population, with women developing the disease three times more frequently than men.[1] Interestingly, a spontaneous improvement of RA has been observed for many patients during pregnancy with a flare after delivery.[2] Pregnancy is the only natural situation known to show this effect. Various immunomodulatory processes have been described during pregnancy in order to accommodate the fetus, e.g. (local) influx of T regulatory cells and regulation of cytokine and receptor expression, as reviewed in refs 3−5, among others.

Antibodies are key players in the immune system, protecting the body from a large variety of threats. In autoimmune diseases, like RA, antibodies against self-antigens, autoantibodies for short, are thought to represent an important pathogenic mechanism.[6] The most abundant antibody in the human circulation is immunoglobulin G (IgG). IgG autoantibodies are present in patients with RA.[7] IgGs are glycoproteins carrying predominantly biantennary complex-type N-glycans in the constant region of the heavy polypeptide chains of the fragment crystallizable (Fc) domain. This glycan contains a heptasaccharide core which may carry a core fucose, galactose(s), sialic acid(s) (SA) and/or a (bisecting) N-acetylglucosamine (GlcNAc) (Figure 1A).[8]

IgG Fc glycosylation features strongly associate with the pro- and anti-inflammatory properties of IgG.[8−14] However, a clear functional role for the IgG Fc glycosylation in determining disease activity in autoimmune diseases has not been demonstrated yet. It has been suggested that IgG Fc glycosylation changes play a role in pregnancy induced remission of RA.[15] Pregnancy could, therefore, provide a

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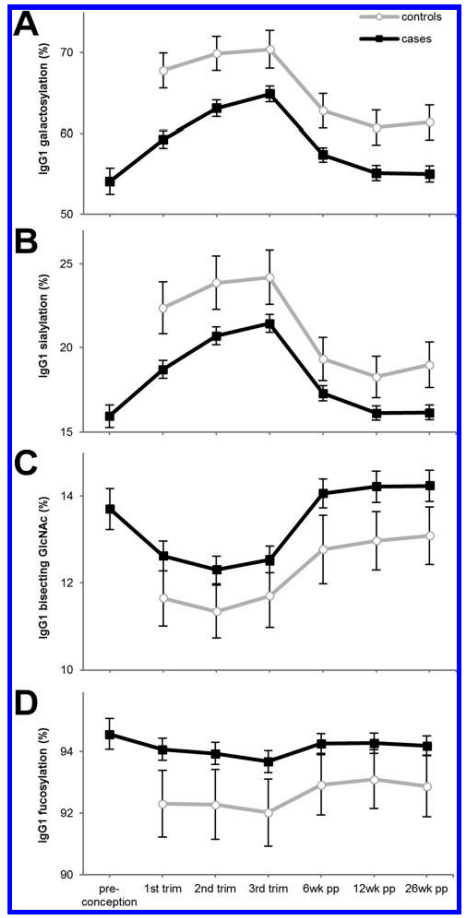


Figure 2. IgG1 Fc-glycosylation changes during pregnancy and after delivery. Galactosylation (A; $p < 0.0001$), sialylation (B; $p < 0.0001$), incidence of bisecting GlcNAc (C), and fucosylation (D; $p < 0.01$) are given for RA patients (black) and healthy controls (gray). Error bars represent the 95% confidence intervals. Abbreviations: GlcNAc, N-acetylglucosamine; trim, trimester of pregnancy; wk, weeks; and pp, postpartum.

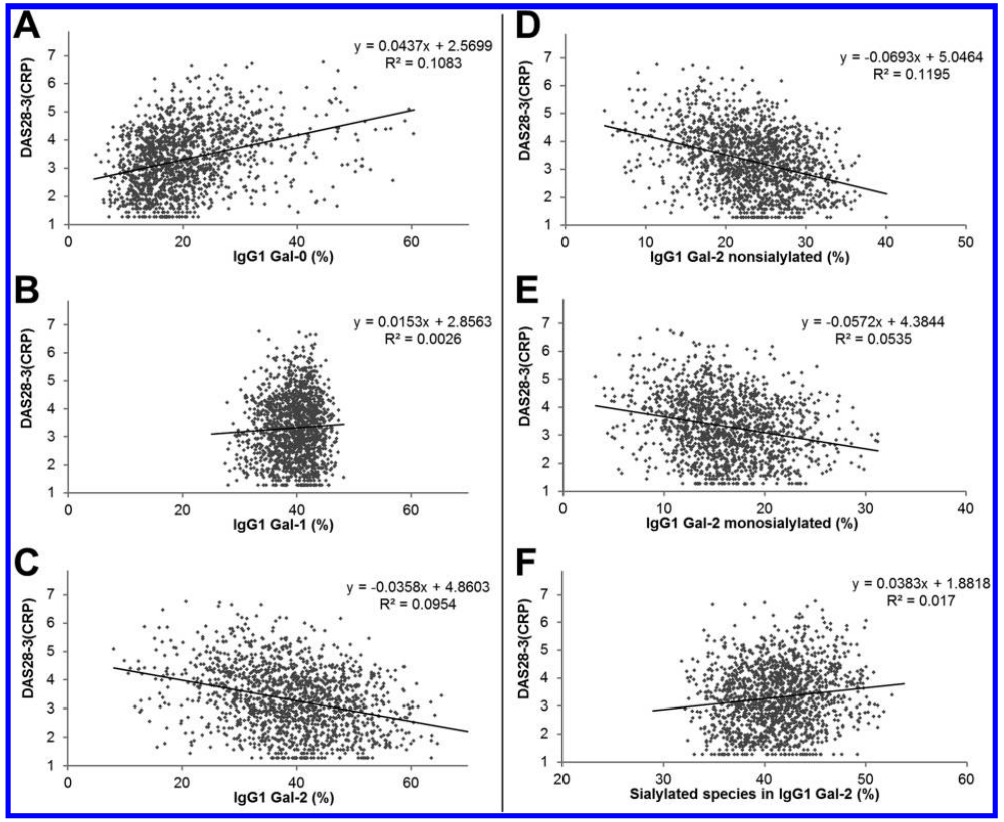
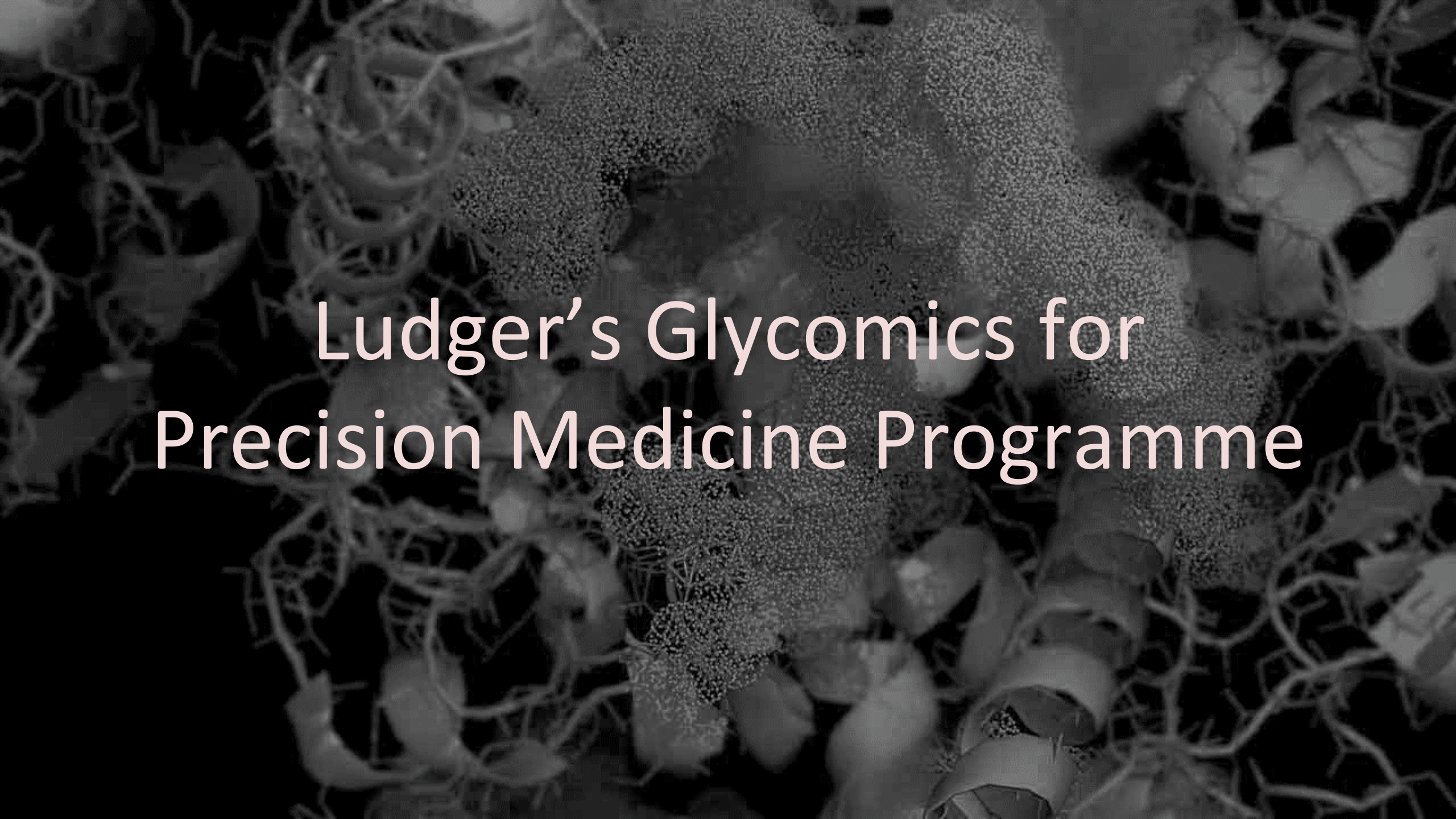
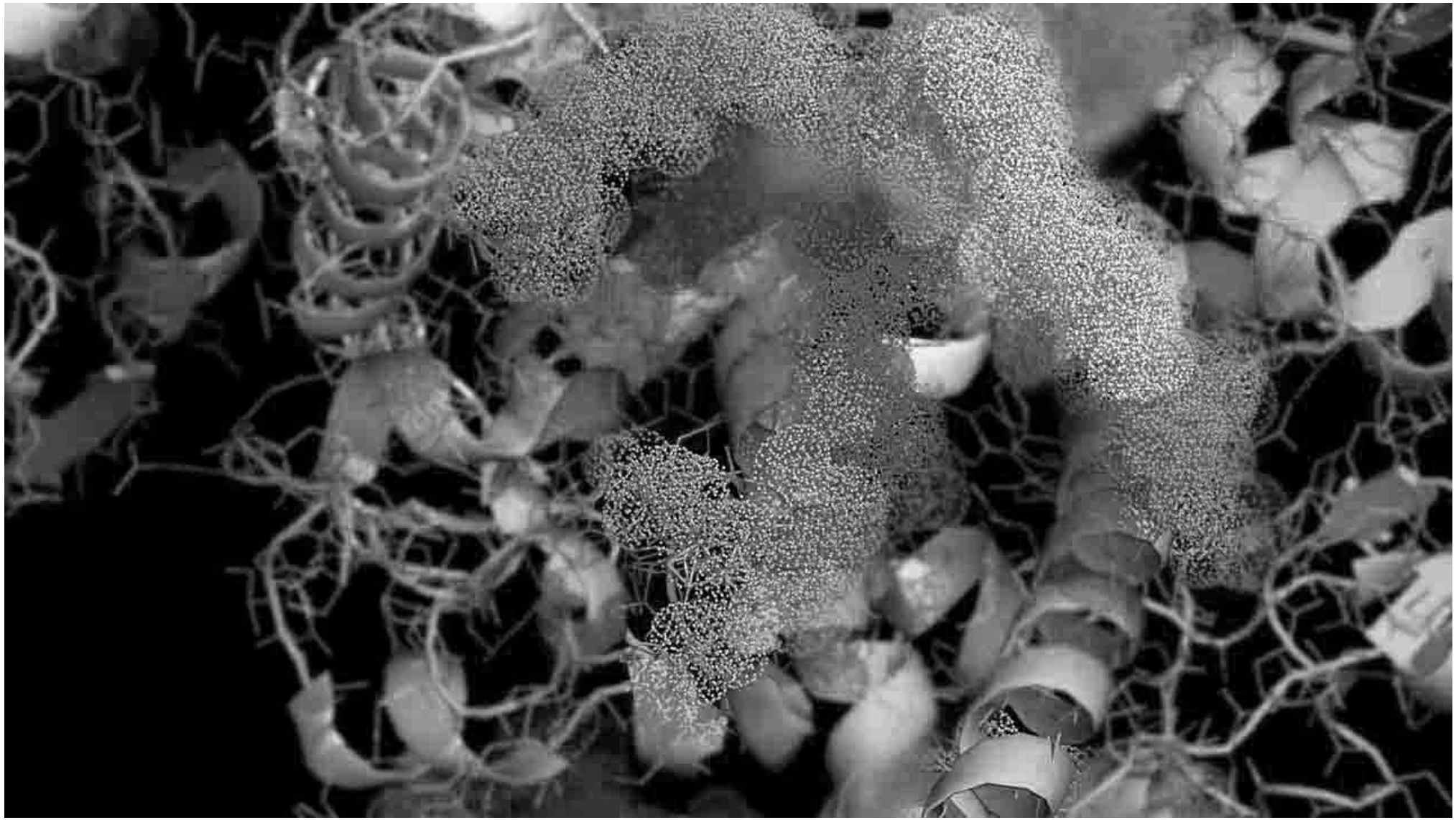


Figure 4. Galactosylation is strongly associated with disease activity. The association of disease activity with agalactosylated, monogalactosylated and digalactosylated IgG1 glycoforms is shown (A−C); $p < 0.0001$, $p < 0.06$, $p < 0.0001$, respectively). To determine the effect of sialylation on the association between disease activity and digalactosylation (C), this association was determined separately for nonsialylated IgG1 Gal-2 (D) and monosialylated IgG1 Gal-2 (E), showing in both cases a negative association with disease activity. Note the larger R^2 for the nonsialylated species (D) compared to sialylated species (E). This is further illustrated in F, which demonstrates a minor but positive association of IgG Gal-2 sialylation with disease activity ($p < 0.0001$). Abbreviations: DAS28-3(CRP) = disease activity score based on three variables including CRP; Gal-0 = N-glycan carrying no galactose; Gal-1 = N-glycan carrying one galactose; Gal-2 = N-glycan carrying two galactoses.


Ludger’s Glycomics for
Precision Medicine Programme

Glycosylation is immensely complex and changes with state of health and disease

The human glycome is at least three orders of magnitude (1000x) greater complexity than the genome



Glycosylation signatures can help with patient stratification in many medical conditions

Glycan changes can occur in early onset of many inflammatory diseases, cancers and cardiovascular diseases

SCIENTIFIC REPORTS

OPEN

Glycosylation of plasma IgG in colorectal cancer prognosis

Evropi Theodoratou[1,2,*], Kujtim Thaçi[3,*], Felix Agakov[4], Maria N. Timofeeva[2], Jerko Štambuk[3], Maja Pučić-Baković[3], Frano Vučković[3], Peter Orchard[4], Anna Agakova[4], Farhat V. N. Din[2], Ewan Brown[5], Pauline M. Rudd[6], Susan M. Farrington[2], Malcolm G. Dunlop[2], Harry Campbell[1,2,*] & Gordan Lauc[3,7,*]

Received: 09 February 2016
Accepted: 27 May 2016
Published: 15 June 2016

In this study we demonstrate the potential value of Immunoglobulin G (IgG) glycosylation as a novel prognostic biomarker of colorectal cancer (CRC). We analysed plasma IgG glycans in 1229 CRC patients and correlated with survival outcomes. We assessed the predictive value of clinical algorithms and compared this to algorithms that also included glycan predictors. Decreased galactosylation, decreased sialylation (of fucosylated IgG glycan structures) and increased bisecting GlcNAc in IgG glycan structures were strongly associated with all-cause ($q < 0.01$) and CRC mortality ($q = 0.04$ for galactosylation and sialylation). Clinical algorithms showed good prediction of all-cause and CRC mortality (Harrell's C: 0.73, 0.77; AUC: 0.75, 0.79, IDI: 0.02, 0.04 respectively). The inclusion of IgG glycan data did not lead to any statistically significant improvements overall, but it improved the prediction over clinical models for stage 4 patients with the shortest follow-up time until death, with the median gain in the test AUC of 0.08. These glycan differences are consistent with significantly increased IgG pro-inflammatory activity being associated with poorer CRC prognosis, especially in late stage CRC. In the absence of validated biomarkers to improve upon prognostic information from existing clinicopathological factors, the potential of these novel IgG glycan biomarkers merits further investigation.

Theodoratou et al (2016) Scientific Reports 6:28098. PMC4908421

Research Article

The Glycosylation of AGP and Its Associations with the Binding to Methadone

Jennifer L. Behan,[1] Yvonne E. Cruickshank,[1] Gerri Matthews-Smith,[2] Malcolm Bruce,[2] and Kevin D. Smith[1,3]

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[2] *School of Nursing, Midwifery and Social Care, Edinburgh Napier University, Edinburgh EH11 4BN, UK*
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Received 3 April 2013; Revised 21 June 2013; Accepted 21 June 2013

Academic Editor: Viness Pillay

Methadone remains the most common form of pharmacological therapy for opioid dependence; however, there is a lack of explanation for the reports of its relatively low success rate in achieving complete abstinence. One hypothesis is that *in vivo* binding of methadone to the plasma glycoprotein alpha-1-acid glycoprotein (AGP), to a degree dependent on the molecular structure, may render the drug inactive. This study sought to determine whether alterations present in the glycosylation pattern of AGP in patients undergoing various stages of methadone therapy (titration < two weeks, harm reduction < one year, long-term > one and a half years) could affect the affinity of the glycoprotein to bind methadone. The composition of AGP glycosylation was determined using high pH anion exchange chromatography (HPAEC) and intrinsic fluorescence analysed to determine the extent of binding to methadone. The monosaccharides galactose and N-acetyl-glucosamine were elevated in all methadone treatment groups indicating alterations in AGP glycosylation. AGP from all patients receiving methadone therapy exhibited a greater degree of binding than the normal population. This suggests that analysing the glycosylation of AGP in patients receiving methadone may aid in determining whether the therapy is likely to be effective.

Behan et al (2013) Biomed Res Int. 2013:108902.. PMC4908421

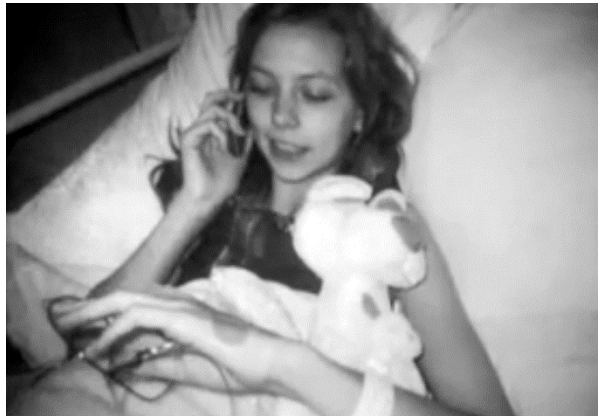
Positioning of Glycomics for Personalised Medicine

Glycomics and genomics are complementary tests for PM

Personalised Medicine

Patient Stratification

Clinical Treatment



Based on the particular patient and their biomolecular dysfunction

Genomics

Disease Possibility

GENETests™
GENETESTS.ORG

Nov 13, 2016

63,971 Tests

4,715 Disorders

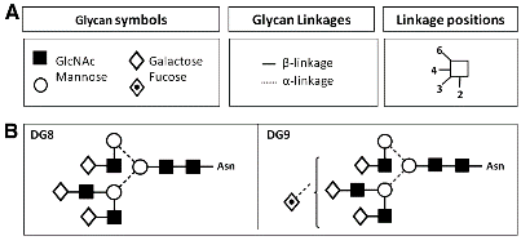
5,669 Genes

697 Laboratories

1,080 Clinics

Glycomics

Disease Actuality



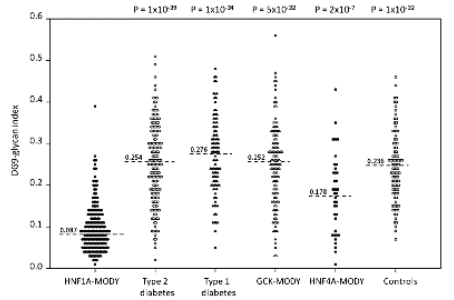


FIG. 2. Dot histograms illustrating the DG9-glycan index in different diabetes subtypes and nondiabetic control subjects. Subjects are represented by the following symbols: ● = *HNF1A*-MODY; ○ = type 2 diabetes; ▲ = type 1 diabetes; △ = *GCK*-MODY (*GCK*-MODY); ■ = *HNF4A*-MODY (*HNF4A*-MODY); | = nondiabetic controls. *P* values are calculated by Mann–Whitney *U* tests in comparison with subjects with *HNF1A*-MODY. The median value of the DG9-glycan index for each diabetes subtype is highlighted adjacent to a black dashed line.

2 Tests

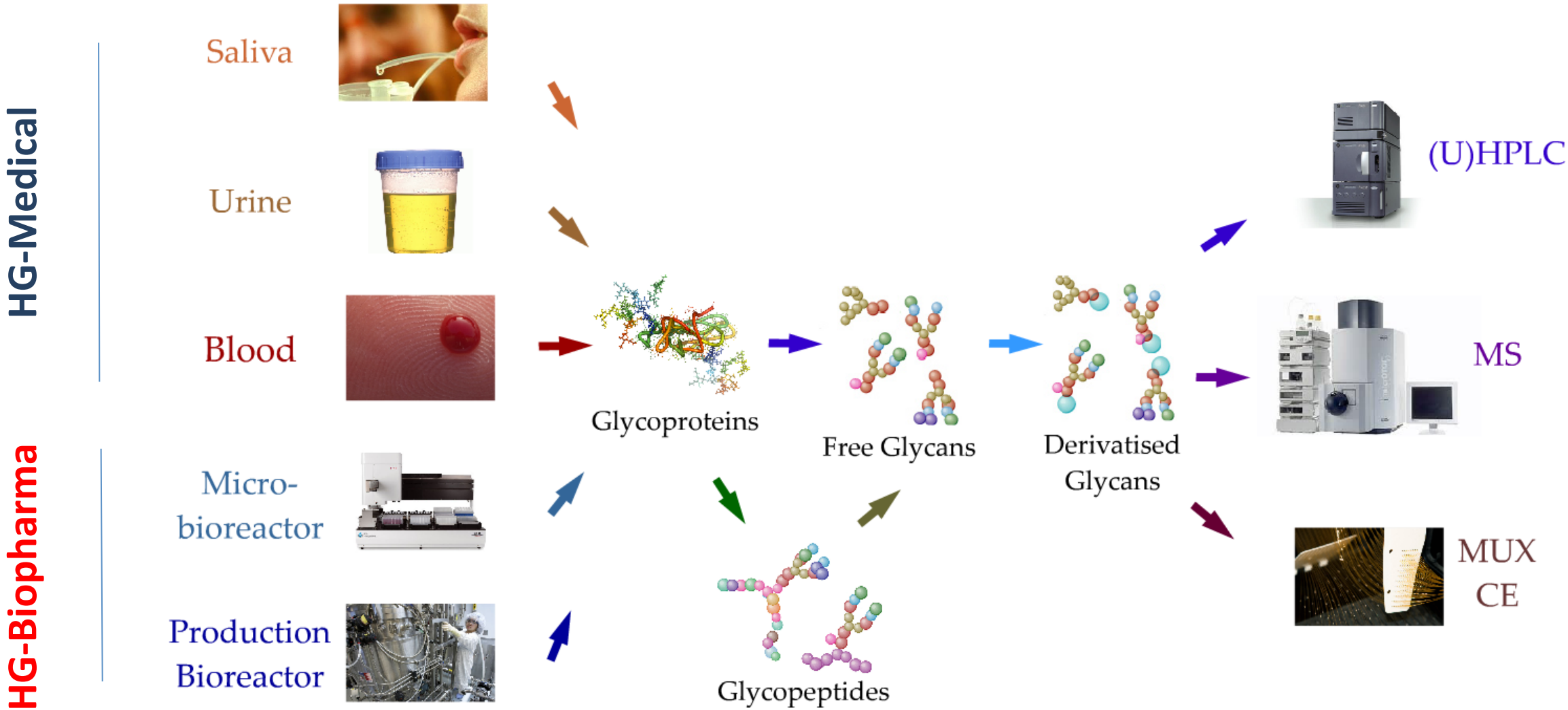
Ludger Glycomics Lab, 2016

Ludger – Science and Business Staff at Culham Science Centre Site

Ludger staff in Sept 2016: 26 staff in UK, 2 sales people in China, 4 regular consultants

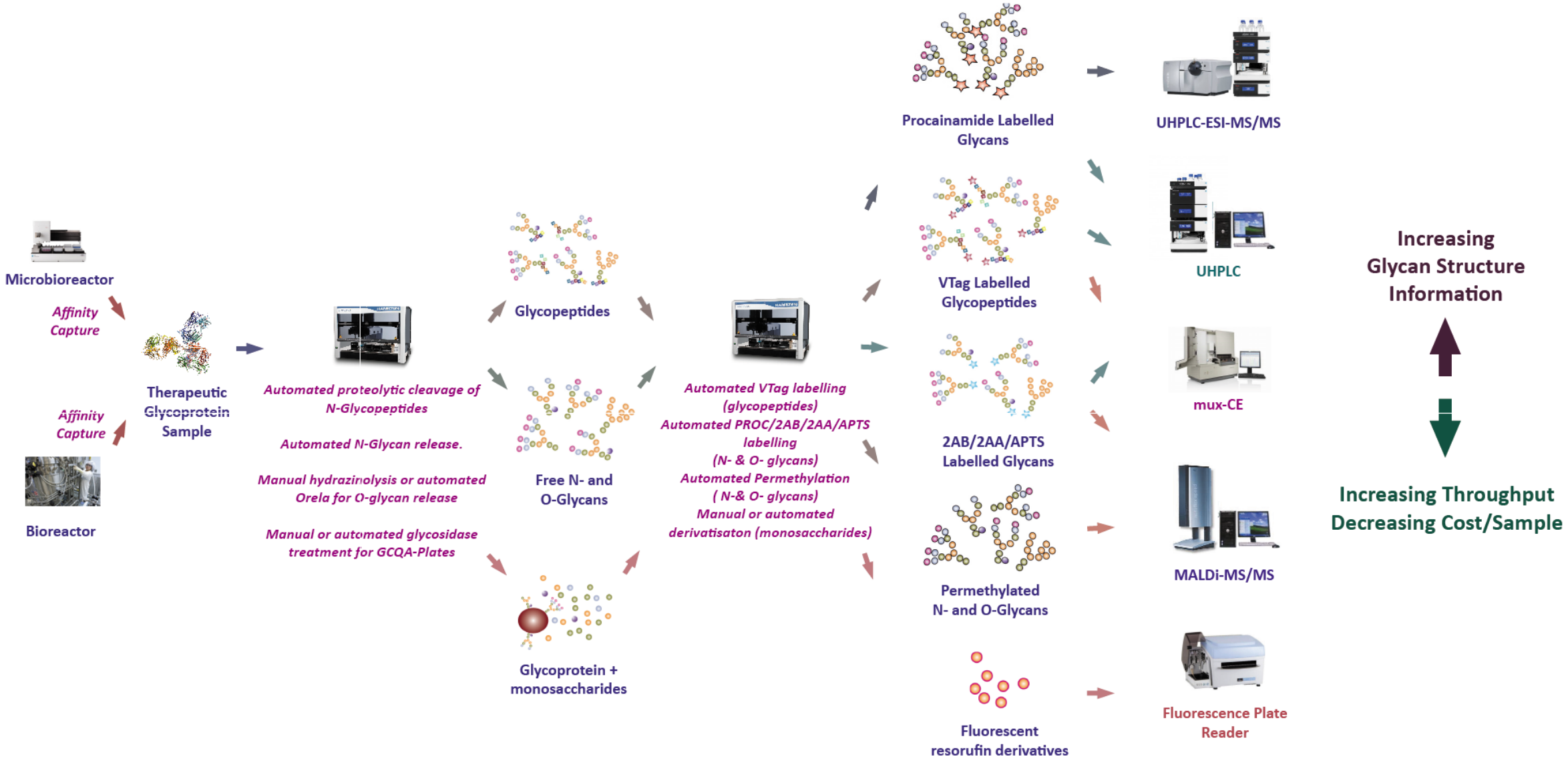
Original concept for HighGlycan HT glycomics workflows

HighGlycan is a €6M, 5 year EU FP7 funded programme to develop high throughput glycomics methods

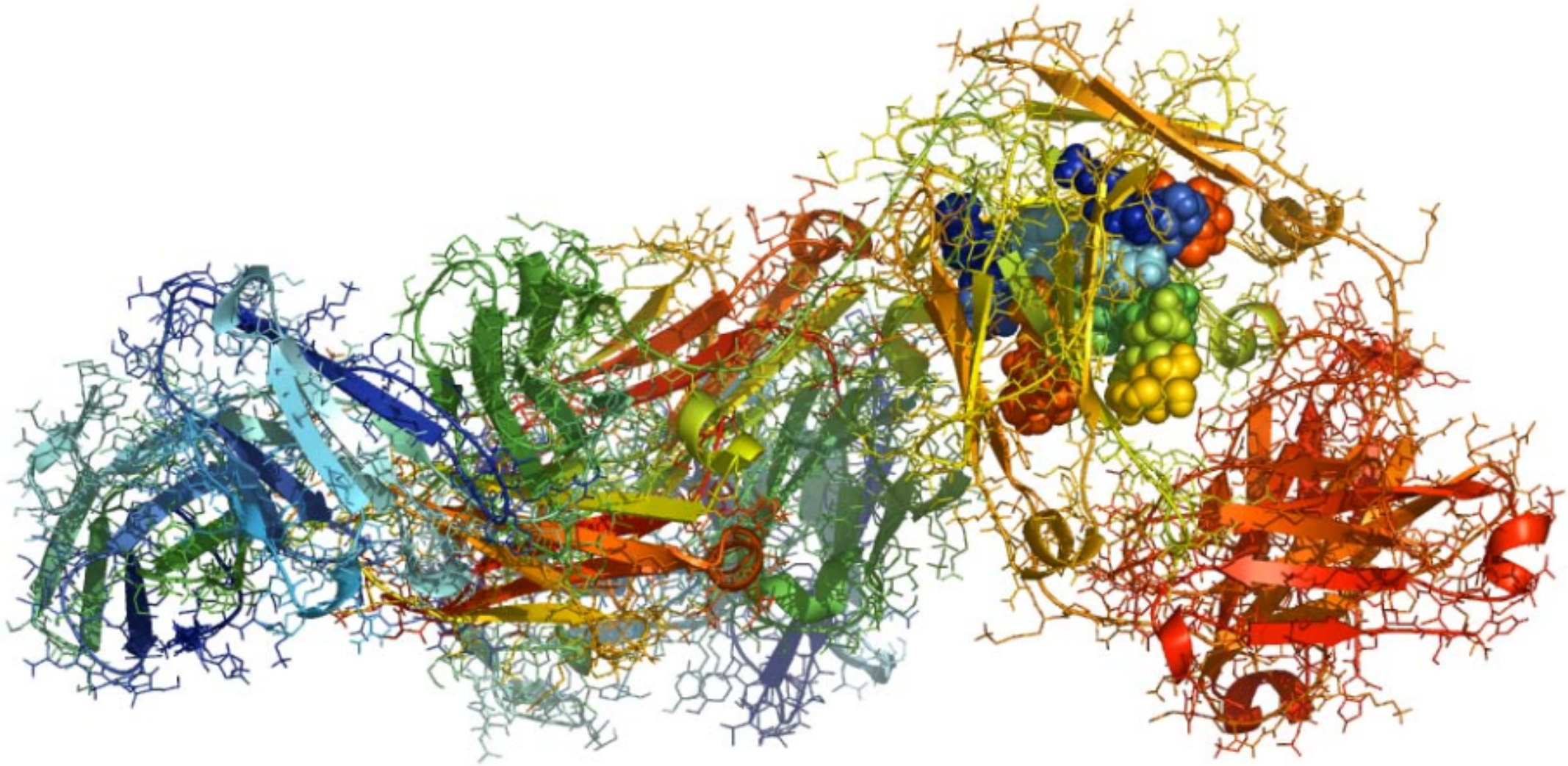


The LongBow™ Glycomics Engine: Ludger's implementation of the HighGlycan Concept

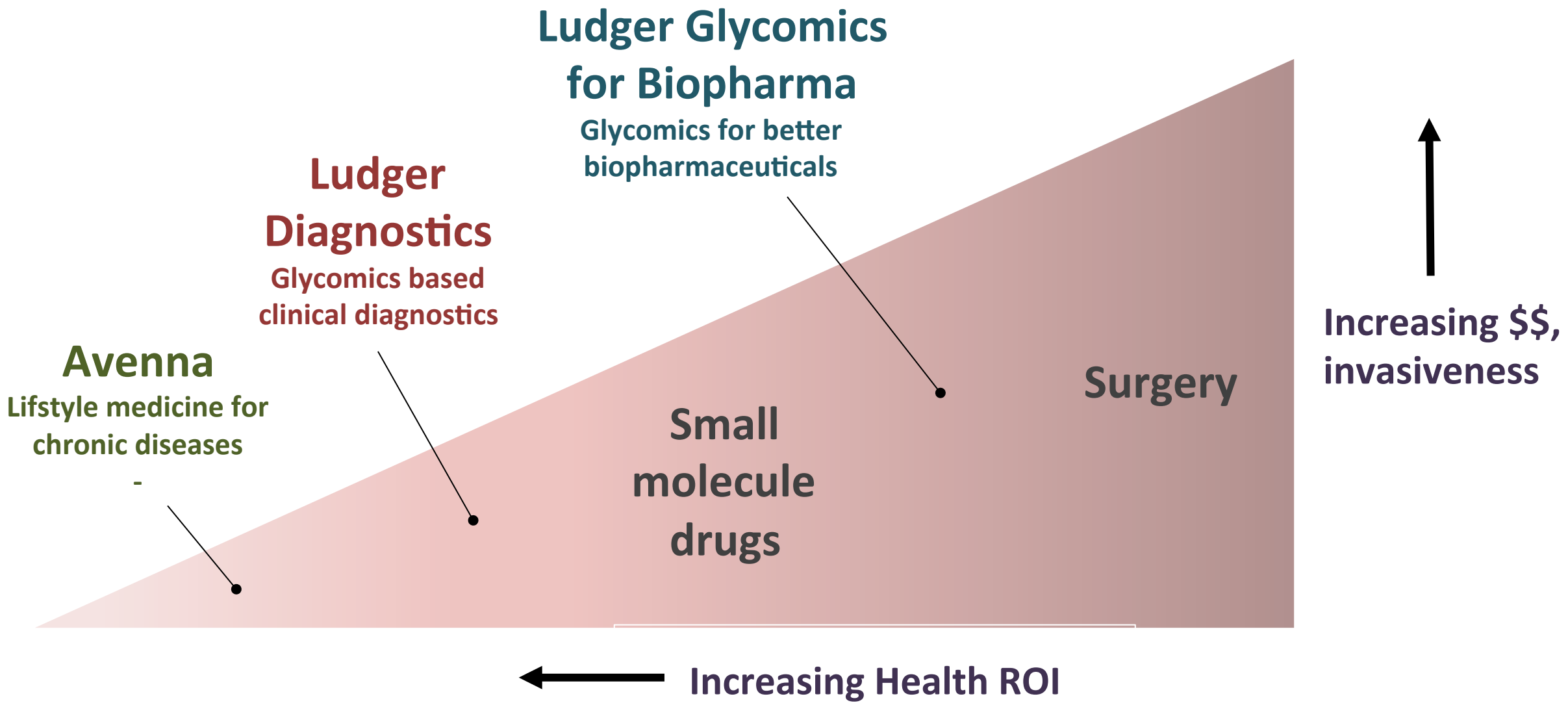
Allows larger, faster, more detailed glycomics studies at lower cost per sample



Focus on blood glycoproteins, particularly IgG

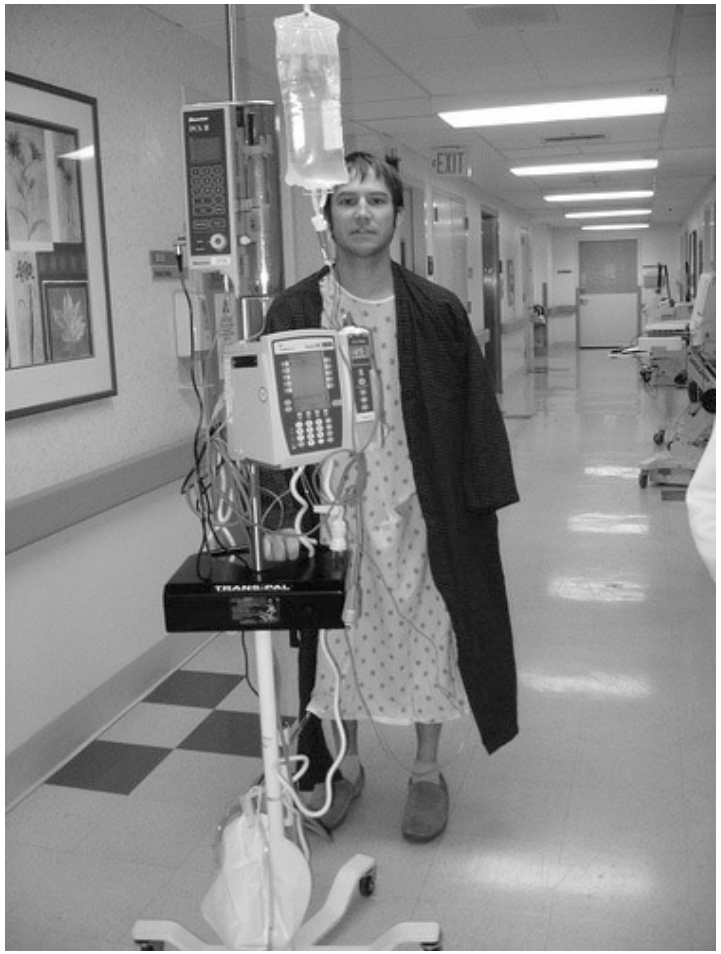


Mapping Ludger businesses onto the Healthcare Cost Effectiveness Wedge



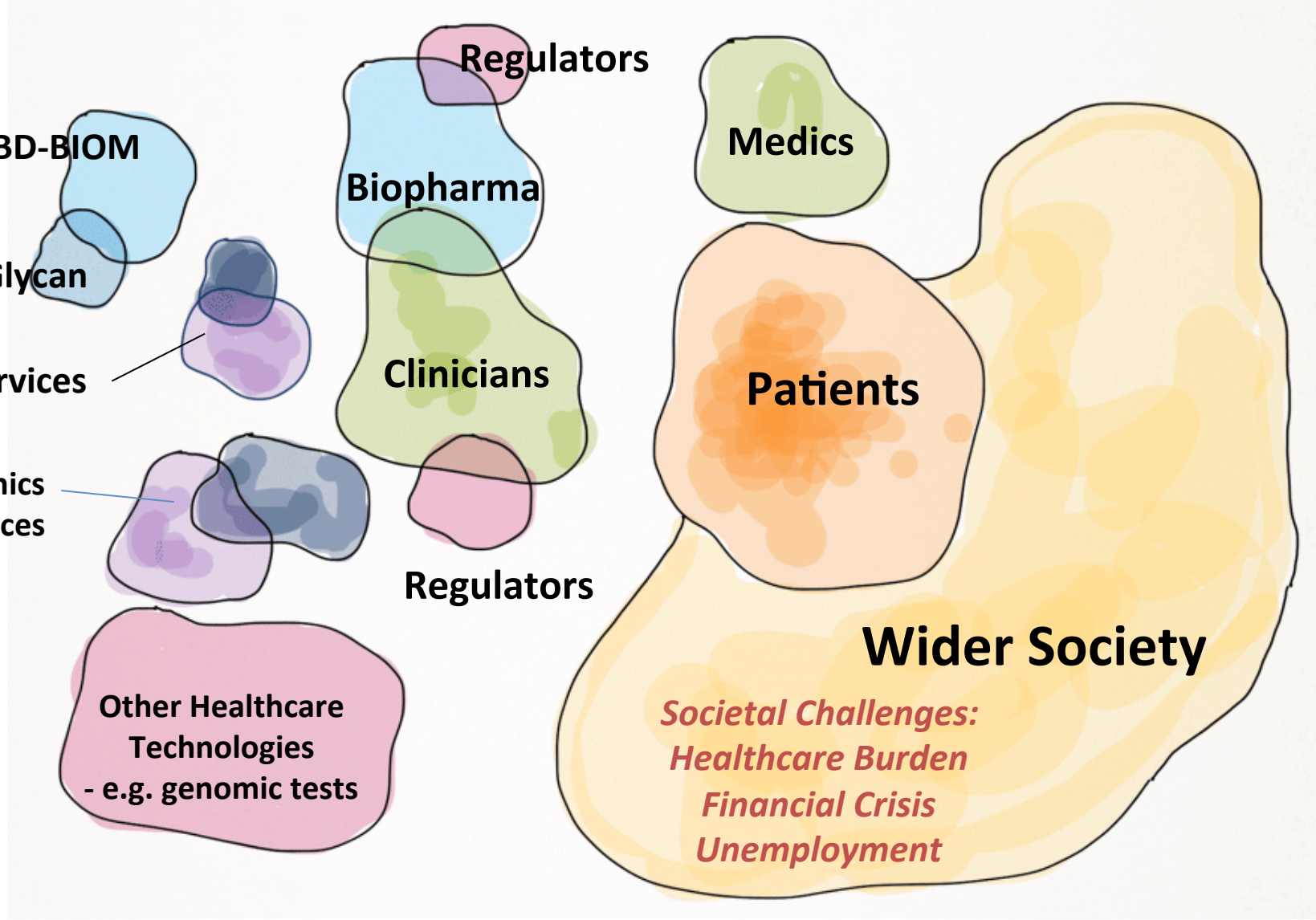
临床诊断:炎症性肠病经常停留在多年未确诊

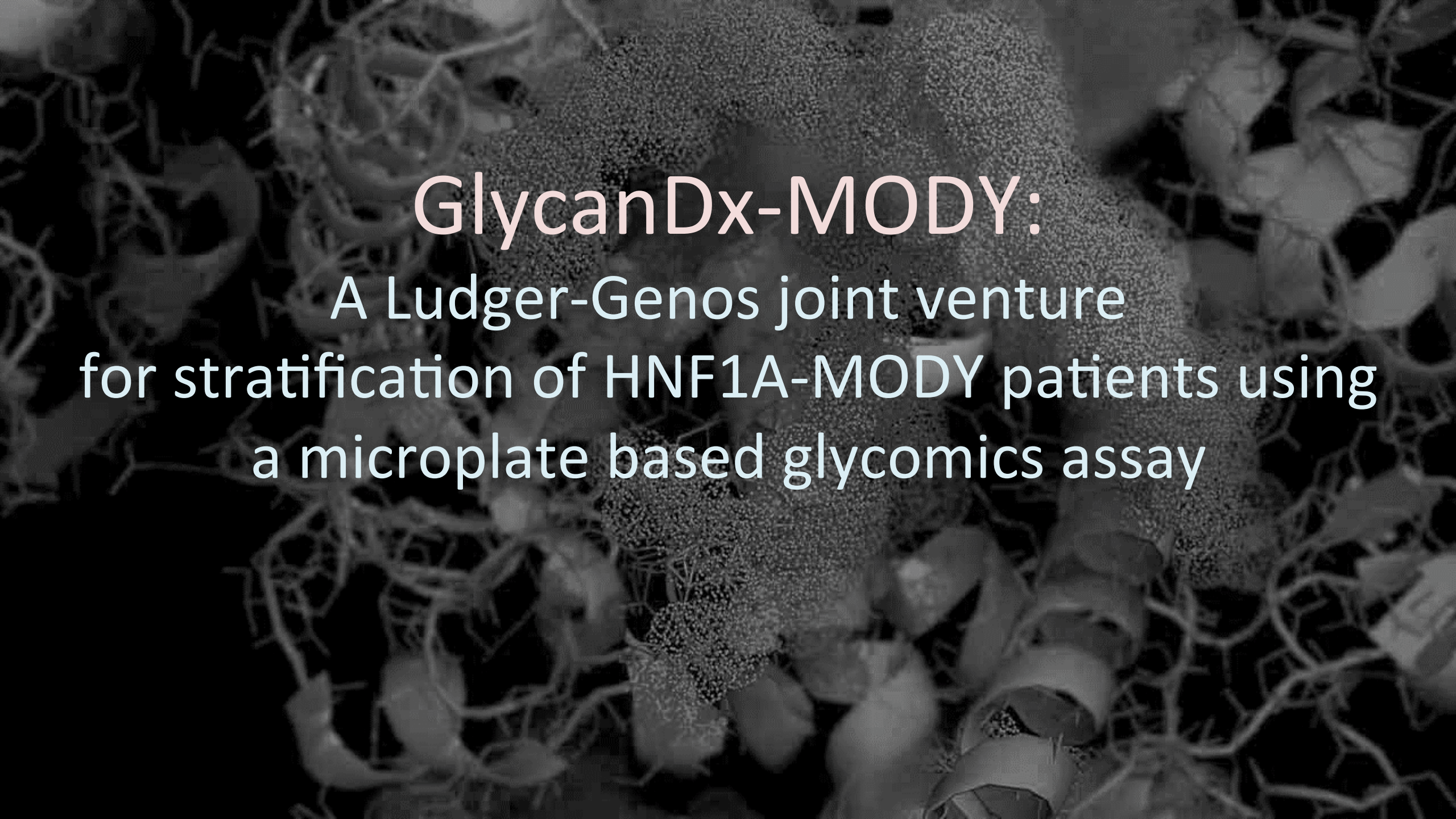
Clinical diagnostics: Inflammatory bowel diseases often remain undiagnosed for years



Mark's IBD Surgery and Stumpy the Stoma: http://ucstory.wordpress.com/surgery-1/

How HighGlycan fits into the Healthcare Ecosystem




GlycanDx-MODY:
A Ludger-Genos joint venture
for stratification of HNF1A-MODY patients using
a microplate based glycomics assay

Ludger partnership with Prof Gordan Lauc, Univ Zagreb and CEO of Genos

Prof. Gordan Lauc

Ludger GENOS

Collaborative Research Projects

HIGH GLYCAN

IBDBIOM

GlyCoCan

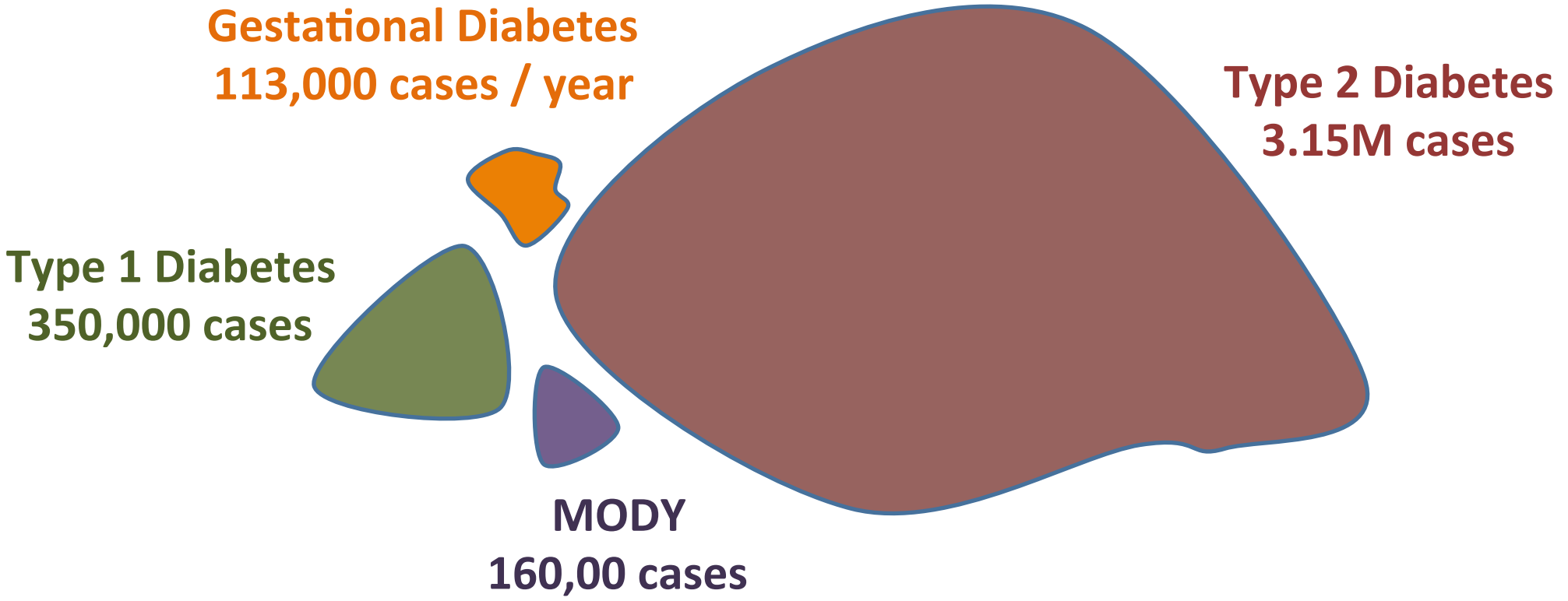
GlySign Glycosylation Signatures for Precision Medicine

GLYCANAGE®

GlycanDx-MODY

Diabetes is a syndrome of diseases with different molecular aetiologies

Patient stratification requires identification of the different aetiological subtypes



Estimated prevalence of different diabetes types in UK

MODY: UK Stats

Misdiagnosis of MODY wastes NHS money and causes uneccessary patient suffering

£9.8bn (2015) **£16.9bn** (2035)

Annual NHS Spend on Diabetes Treatment

Up to **160,000** individuals

HNF1A-MODY Prevalence (1-4% diabetics in UK)

~ 90%

MODY patients misdiagnosed

~ 70%

MODY patients with HNF1A-MODY

Blindness, kidney disease, microvascular complications

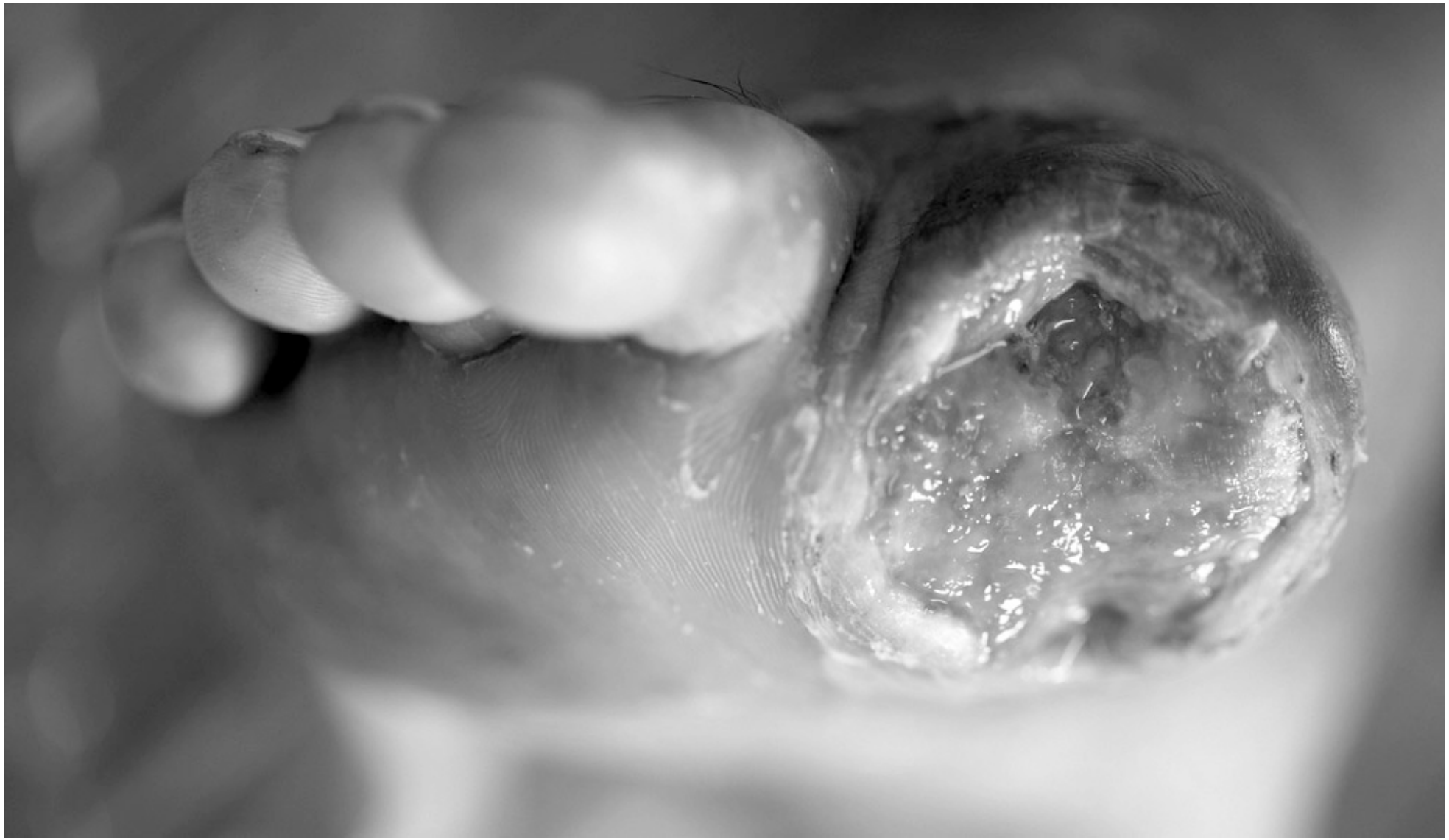
Long Term Complications due to poor glycaemic control

~ £400/patient/yr

Savings on meds from insulin to low dose oral sulfonylurea drugs

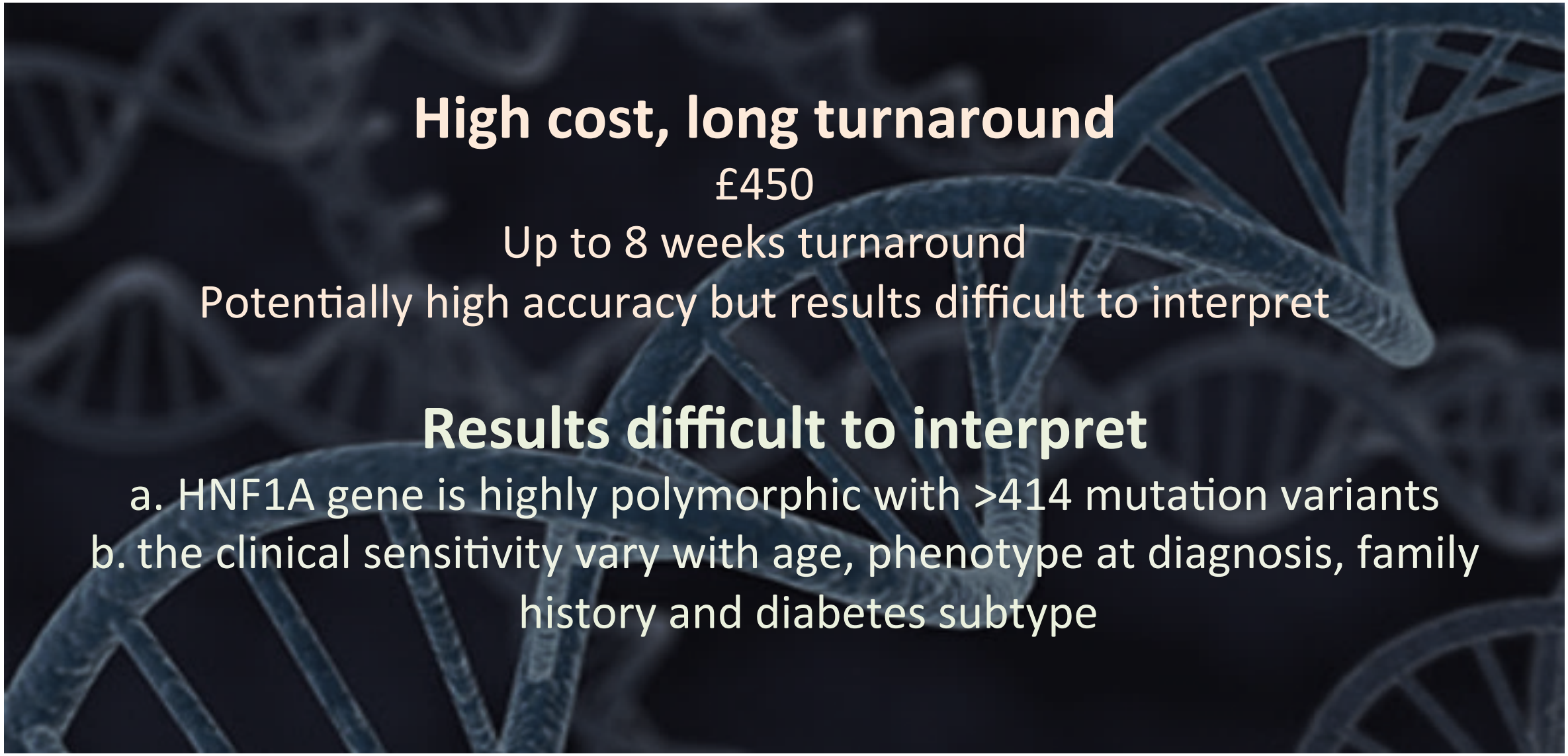
Misdiagnosed HNF1A-MODY patients suffer and waste NHS money

The financial burden is probably greatly disproportional to the prevalence of HNF1A-MODY



MODY Gene Test

Why it isn't practical for primary health care



High cost, long turnaround

£450

Up to 8 weeks turnaround

Potentially high accuracy but results difficult to interpret

Results difficult to interpret

a. HNF1A gene is highly polymorphic with >414 mutation variants

b. the clinical sensitivity vary with age, phenotype at diagnosis, family history and diabetes subtype

Lauc patent: Outer arm fucosylation as a biomarker of HNF1A-MODY

Mutations in HNF1A regulator gene disrupts glycaemic control as well fucosylation of plasma glycoproteins

Plasma N-Glycosylation changes in MODY-type diabetes

ORIGINAL ARTICLE

Mutations in *HNF1A* Result in Marked Alterations of Plasma Glycan Profile

Gaya Thanabalasingham,[1,2] Jennifer E. Huffman,[3] Jayesh J. Kattla,[4] Mislav Novokmet,[5] Igor Rudan,[6,7] Anna L. Gloyn,[1,2] Caroline Hayward,[3] Barbara Adamczyk,[4] Rebecca M. Reynolds,[8] Ana Muzinic,[5] Neelam Hassanali,[1] Maja Pucic,[5] Amanda J. Bennett,[1] Abdelkader Essafi,[3] Ozren Polasek,[7] Saima A. Mughal,[1,2] Irma Redzic,[9] Dragan Primorac,[7,10] Lina Zgaga,[6] Ivana Kolcic,[7] Torben Hansen,[11,12,13] Daniela Gasperikova,[14] Erling Tjora,[15,16] Mark W.J. Strachan,[17] Trine Nielsen,[11] Juraj Stanik,[14,18] Iwar Klimes,[14] Olaf B. Pedersen,[11,19,20] Pål R. Njølstad,[15,16] Sarah H. Wild,[8] Ulf Gyllensten,[21] Olga Gornik,[5] James F. Wilson,[6] Nicholas D. Hastie,[3] Harry Campbell,[8] Mark I. McCarthy,[1,2,22] Pauline M. Rudd,[4] Katharine R. Owen,[1,2] Gordan Lauc,[5,9] and Alan F. Wright[3]

A recent genome-wide association study identified hepatocyte nuclear factor 1-α (*HNF1A*) as a key regulator of fucosylation. We hypothesized that loss-of-function *HNF1A* mutations caused by maturity-onset diabetes of the young (MODY) would display altered fucosylation of *N*-linked glycans on plasma proteins and that glycan biomarkers could improve the efficiency of a diagnosis of *HNF1A*-MODY. In a pilot comparison of 43 subjects with *HNF1A*-MODY and 41 subjects with type 2 diabetes, 15 of 29 glycan measurements differed between the two groups. The DG9-glycan index, which is the ratio of fucosylated to nonfucosylated triantennary glycans, provided optimum discrimination in the pilot study and was examined further among additional subjects with *HNF1A*-MODY (n = 188), glucokinase (*GCK*)-MODY (n = 118), hepatocyte nuclear factor 4-α (*HNF4A*)-MODY (n = 40), type 1 diabetes (n = 393), type 2 diabetes (n = 467), and nondiabetic controls (n = 98). The DG9-glycan index was markedly lower in *HNF1A* MODY than in controls or other diabetes subtypes, offered good discrimination between *HNF1A*-MODY and both type 1 and type 2 diabetes (*C* statistic >0.90), and enabled us to detect three previously undetected *HNF1A* mutations in patients with diabetes. In contrast, glycan profiles are altered substantially in *HNF1A*-MODY and the DG9-glycan index has potential clinical value as a diagnostic biomarker of *HNF1A* dysfunction. ***Diabetes*** **62:1329–1337, 2013**

Genome wide association studies are providing novel insights into the genetic architecture and biological basis of many diseases, but immediate translation into clinical practice has been limited. We recently performed a genome wide association study of the human plasma *N* glycome and found evidence of association involving common variants near the hepatocyte nuclear factor 1-α (*HNF1A*) gene; follow-up functional experiments established HNF1A as a master regulator of plasma protein fucosylation (1). Fucosylation, a specific type of glycosylation, comprises the addition of fucose residues to glycans. Here we evaluate the hypothesis that mutations causing a more severe deficit in HNF1A function (resulting in the monogenic subtype of diabetes known as *HNF1A* maturity onset diabetes of the young [MODY; *HNF1A* MODY]) are associated with marked alterations of plasma glycome composition, and we assess the value of glycan profiles as a diagnostic biomarker for *HNF1A* MODY.

Most human proteins are posttranslationally modified by the addition of complex oligosaccharide structures (glycans) (2). Despite the impact on protein structure and function, the clinical consequences of changes in the human glycome remain largely unexplored, primarily because reliable analytical techniques have been developed only recently (3). In recent studies, HNF1A was shown to promote both the de novo and salvage pathways for the synthesis of guanosine diphosphate fucose (1) and to regulate fucosyltransferase VI (1,4). HNF1A thereby controls the outer arm (antennary) fucosylation of proteins with *N*-linked glycans through effects on both the supply of activated precursors and the incorporation of fucose (1,4).

Mutations disrupting *HNF1A* are responsible for the most common subtype of monogenic diabetes, *HNF1A*-MODY (5). Like other forms of MODY, *HNF1A* MODY is characterized

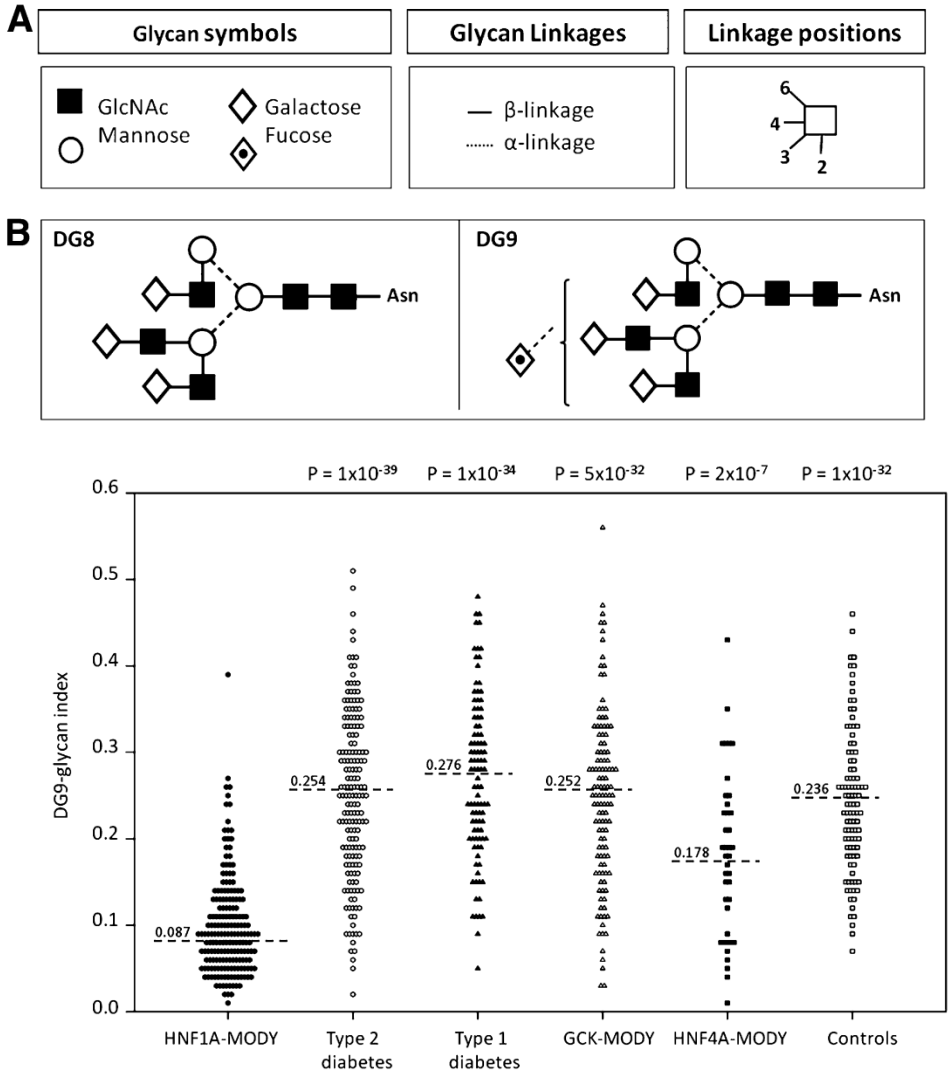
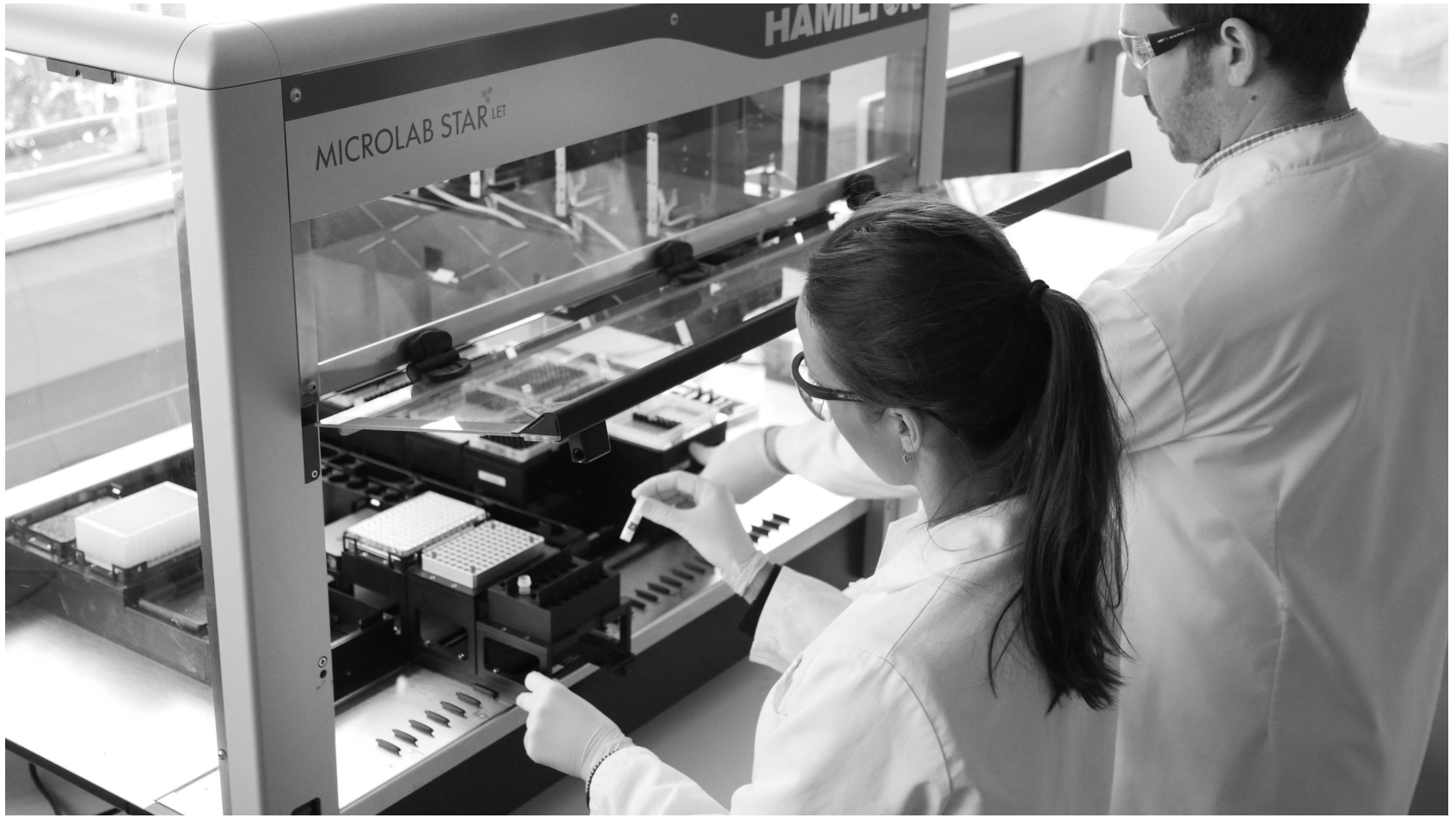


FIG. 2. Dot histograms illustrating the DG9-glycan index in different diabetes subtypes and nondiabetic control subjects. Subjects are represented by the following symbols: ● = *HNF1A*-MODY; ○ = type 2 diabetes; ▲ = type 1 diabetes; △ = *GCK*-MODY (*GCK*-MODY); ■ = *HNF4A*-MODY (*HNF4A*-MODY); □ = nondiabetic controls. *P* values are calculated by Mann-Whitney *U* tests in comparison with subjects with *HNF1A*-MODY. The median value of the DG9-glycan index for each diabetes subtype is highlighted adjacent to a *black dashed line*.

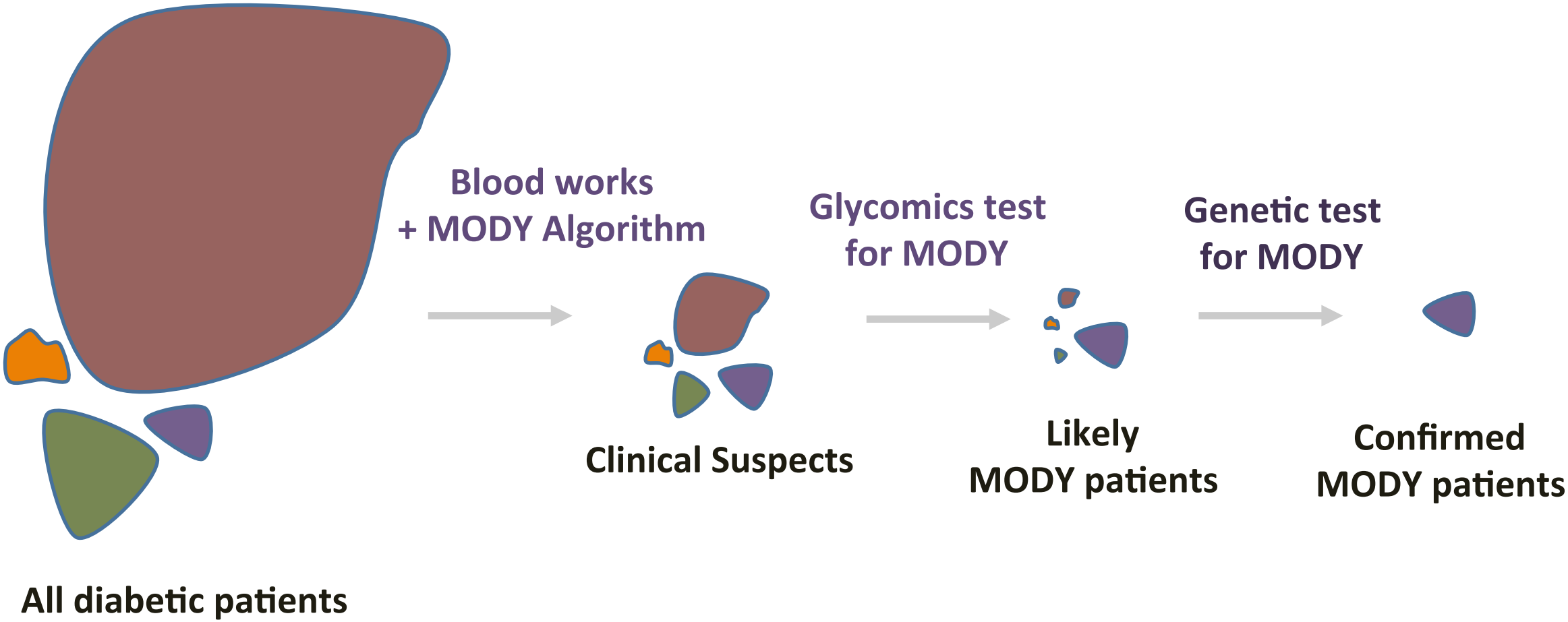
Ludger's GlycanDx-MODY assay exploits the Lauc patent

This is a microtitre plate based assay that is accurate, robust and cheap enough for use as a clinical diagnostic



Precision Medicine Diagnostic Pathway for MODY Diabetes

This is a microtitre plate based assay that is accurate, robust and cheap enough for use as a clinical diagnostic



GlycanDx-MODY Algorithm and Assay

MODY Suspect Algorithm

Free of charge

Use in GP's surgery

Input: Normal blood works

Output: Clinical suspicion probability

Assay is Affordable and Fast

£100 (target price)

3 days turnaround

Assay is Easy to Use and Accurate

Easy to interpret results

ROC AUC = 0.92

GlycanDx-MODY Health Economics for UK

Calculations for Ludger Scenario H1-UK

- UK NHS: ~3.5M diabetics in UK
- Average 3% prevalence of HNF1A-MODY
- PDx-MODY algorithm identifies 20% of diabetics (700K) to be clinical suspects
- GlycanDx-MODY assay is £100
- Cost of testing all clinical suspects = £70M
- Number of MODY patients identified=105,000
- **Average NHS saving on drugs= £400/patient/years. Total drug savings= £42M/year, £420M over 10 years**
- Savings on inpatient care due to misdiagnosis and subsequent long term complications over 30 years= £27k/patient
- **total savings by avoiding long term complications (assuming 10% HNF1A-MODY patients develop LTCs) = £283M/30 years.**
- Cost of GlycanDx-MODY development = £8.5M
- **Total net savings to NHS (less cost of assay development and assay delivery) over 30 years = £1.46bn**
- Annual sales revenue for GlycanDx-MODY service in UK: £7M/yr; Gross profit (@ 60%) = £4.2M/yr

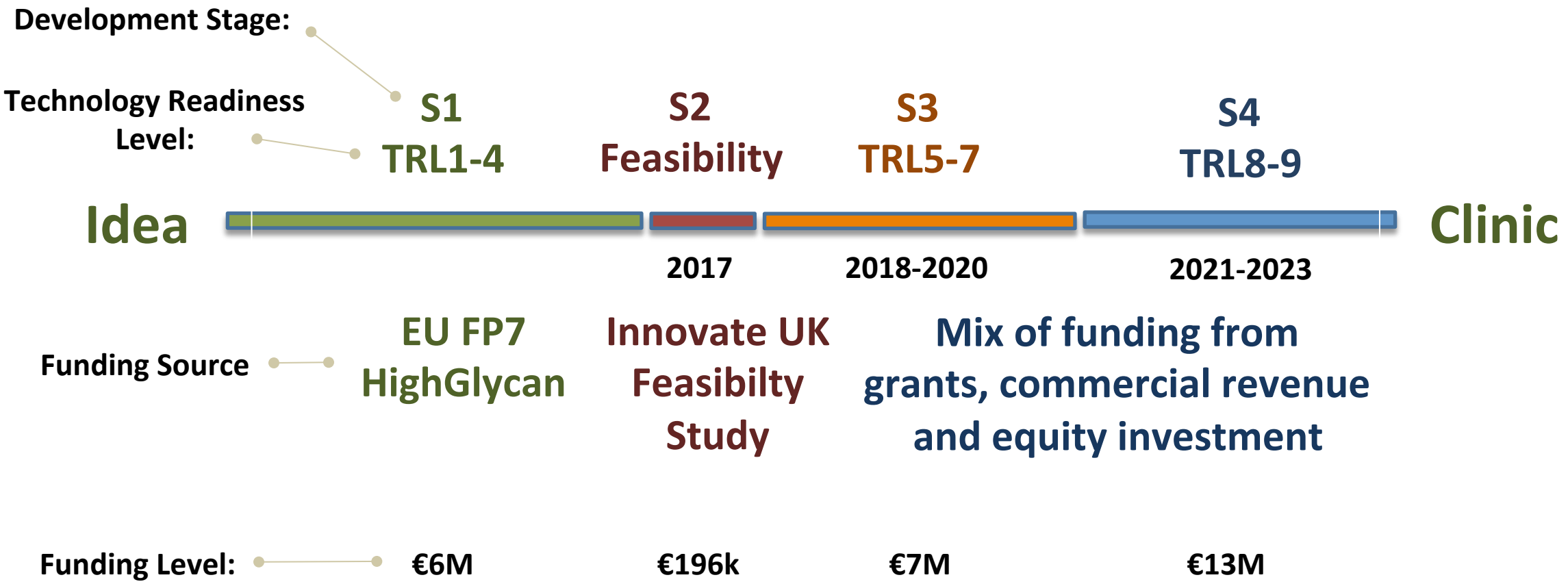
GlycanDx-MODY Health Economics Global Excluding UK

Calculations for Ludger Scenario H1-Global

- 385M diabetic patients worldwide
- Total no. HNF1A-MODY patients, min= 3.85M, max=15.4M
- 2% of diabetic patients are accessible to partner clinical labs per year
- Total no. accessible diabetic patients = 7.7M/yr
- Assume PDx-MODY algorithm identifies 20% as clinical suspects and 40% of those go on to take the GlycoDx-MODY assay
- Total number of tests = 616,00/year;
- Ludger revenue through clinical labs = £30/test;
- Total revenue = £18.4M/year; Gross profit (@80%)=£14.7M/yr

Plan for bringing the GlycanDx-MODY assay to the clinic

JVP between Ludger, Genos and OCDEM

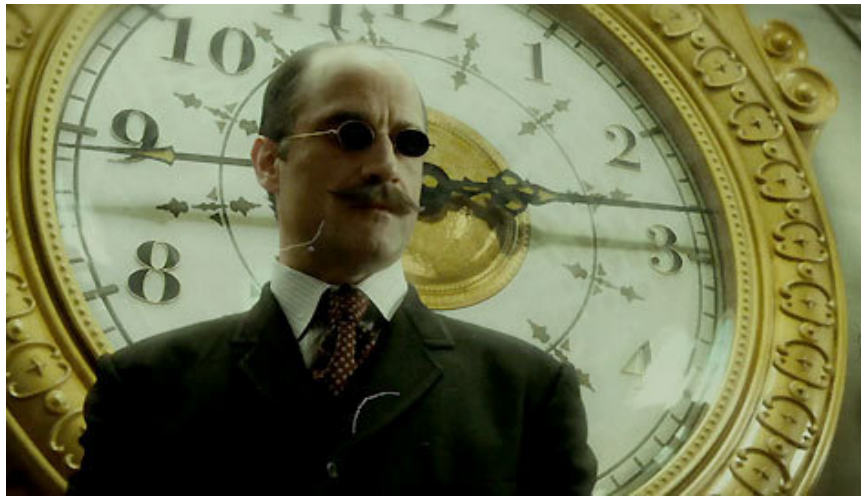



GlycanAge:
A Ludger-Genos joint venture
for determining your hidden biochemical age

Ageing, Death and Disease

Benjamin Button and real life children suffering from Progeria (rapid ageing disease)






GlycanAge Pucić 2011 Paper – 1: IgG Glycome Population Study

Technological Innovations and Resources

⌘ *Author's Choice*

High Throughput Isolation and Glycosylation Analysis of IgG–Variability and Heritability of the IgG Glycome in Three Isolated Human Populations*

Maja Pučić‡, Ana Knežević‡, Jana Vidič§, Barbara Adamczyk¶, Mislav Novokmet‡, Ozren Polašek‖, Olga Gornik**, Sandra Šupraha-Goreta**, Mark R. Wormald‡‡, Irma Redžić**, Harry Campbell§§, Alan Wright¶¶, Nicholas D. Hastie¶¶, James F. Wilson§§, Igor Rudan‖§§, Manfred Wuhrer‖‖, Pauline M. Rudd¶, Djuro Josić[ab], and Gordan Lauc‡**[c]

All immunoglobulin G molecules carry *N*-glycans, which modulate their biological activity. Changes in *N*-glycosylation of IgG associate with various diseases and affect the activity of therapeutic antibodies and intravenous immunoglobulins. We have developed a novel 96-well protein G monolithic plate and used it to rapidly isolate IgG from plasma of 2298 individuals from three isolated human populations. *N*-glycans were released by PNGase F, labeled with 2-aminobenzamide and analyzed by hydrophilic interaction chromatography with fluorescence detection. The majority of the structural features of the IgG glycome were consistent with previous studies, but sialylation was somewhat higher than reported previously. Sialylation was particularly prominent in core fucosylated glycans containing two galactose residues and bisecting GlcNAc where median sialylation level was nearly 80%. Very high variability between individuals was observed, approximately three times higher than in the total plasma glycome. For example, neutral IgG glycans without core fucose varied between 1.3 and 19%, a difference that significantly affects the effector functions of natural antibodies, predisposing or protecting individuals from particular diseases. Heritability of IgG glycans was generally between 30 and 50%. The individual's age was associated with a significant decrease in galactose and increase of bisecting GlcNAc, whereas other functional elements of IgG glycosylation did not change much with age. Gender was not an important predictor for any IgG glycan. An important observation is that competition between glycosyltransferases, which occurs *in vitro*, did not appear to be relevant *in vivo*, indicating that the final glycan structures are not a simple result of competing enzymatic activities, but a carefully regulated outcome designed to meet the prevailing physiological needs. *Molecular & Cellular Proteomics 10: 10.1074/mcp.M111.010090, 1–15, 2011.*

From the ‡Genos Ltd., Glycobiology Division, Planinska 1, 10000

GlycanAge Pucić 2011 Paper – 2: Glycosylation Patterns Change with Age

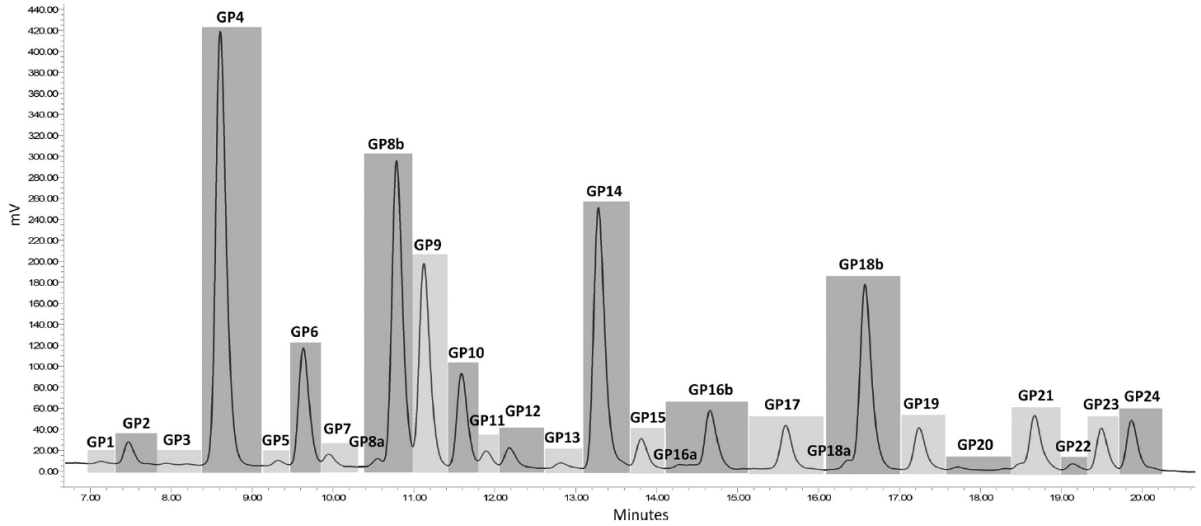


FIG. 1. **UPLC analysis of the IgG glycome.** IgG glycome was separated into 24 chromatographic peaks by hydrophilic interaction chromatography. Compositions and structural schemes of glycans in each chromatographic peak and the average percentage of individual structures are shown in Table I.

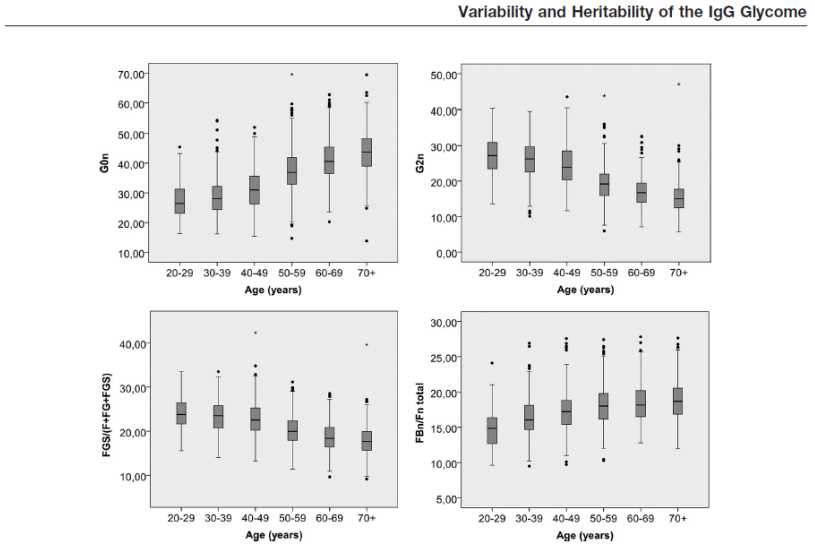


FIG. 2. **Association of IgG glycosylation with age.** Distribution of $G0^n$ glycans, $G2^n$ glycans, the percent of structures with sialic acid (FGS/(F+FG+FGS)) and bisecting GlcNAc ($FB^n/F^{n\ total}$) in fucosylated glycans between different age-groups are shown. Central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value, excluding "outside" and "far out" values that are displayed as separate points.

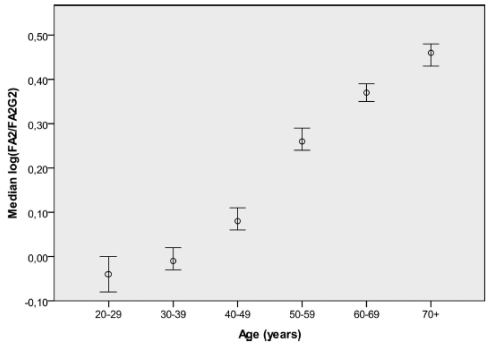


FIG. 3. **The Glyco-Age index.** Glyco-Age index calculated as the logarithm of the ratio of fucosylated G2 and G0 structures (FA2/FA2G2) was recently suggested to be a good indicator of individual's age (78). Median values of the Glyco-Age index (with 95% confidence intervals as error bars) in our study population are shown.

Lauc GlycanAge Patent

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

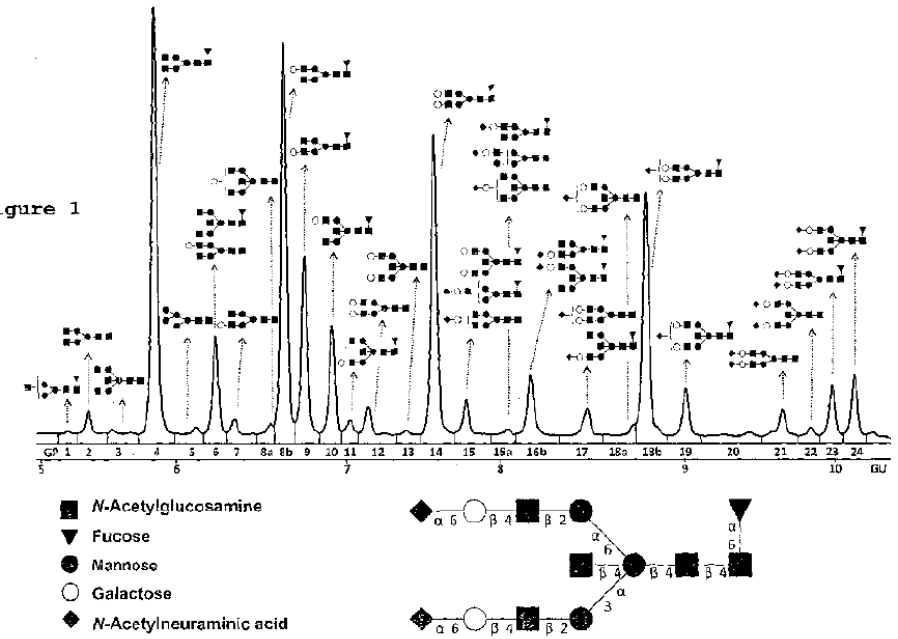
GlycanAge

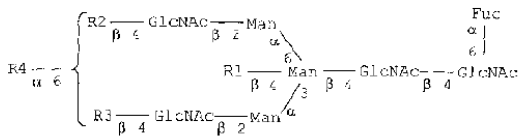
JVP between Ludger and Genos

1/203010 A1

(54) Title: METHOD FOR THE ANALYSIS OF N-GLYCANS ATTACHED TO IMMUNOGLOBULIN G FROM HUMAN BLOOD PLASMA AND ITS USE

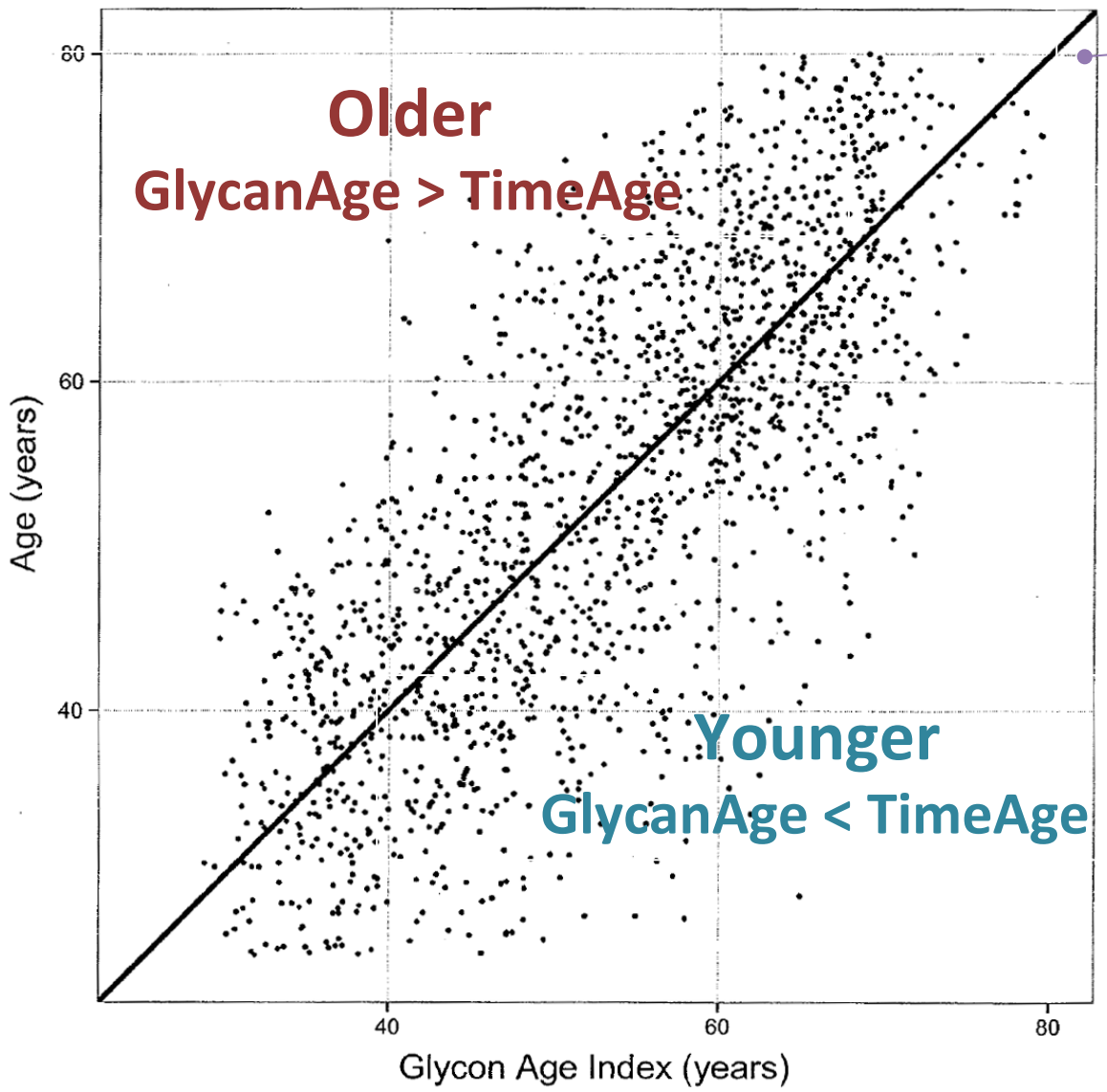
Figure 1





(57) Abstract: The invention discloses a method for the analysis of N-glycans attached to immunoglobulin G (IgG) or IgG N-glycopeptides from human blood plasma in which relative abundance of two or more glycans is determined, out of total six, and for these glycans it is determined that they strongly correlate with age. The glycans have the following structures: F(6) A2 (GP4) : R1, R2, R3, R4= H F (6) A2B (GP6) : R1= GlcNAc; R2, R3, R4 H F(6)A2[6]G1 (GP8) : R1, R3, R4= H; R2= Gal F(6)A2G2 (GP14) : R1= H; R2, R3= Gal; R4= H F(6)A2BG2 (GP15) : R1= GlcNAc; R2, R3= Gal; R4= H F(6)A2G2S1 (GP18): R1= H; R2, R3= Gal; R4= NeuAc GlcNAc = N-acetylglucosamine Fuc = fucose Man = mannose NeuAc = N-acetylneuraminic acid Gal = galactose From the results of the analysis, Glycan Age Index (GAI) is calculated, and it is useful for: prediction of biological age of a tested individual; monitoring efficacy of methods that slow down the ageing process; monitoring progression of diseases that are developed as a result of the ageing process advancement, like: inflammatory diseases (including atherosclerosis), autoimmune diseases, tumours, diabetes, arthritis, osteoporosis, and Alzheimer disease; and evaluation of overall condition/health of a body.

GlycanAge appears to be a reliable indicator of your body's biological age

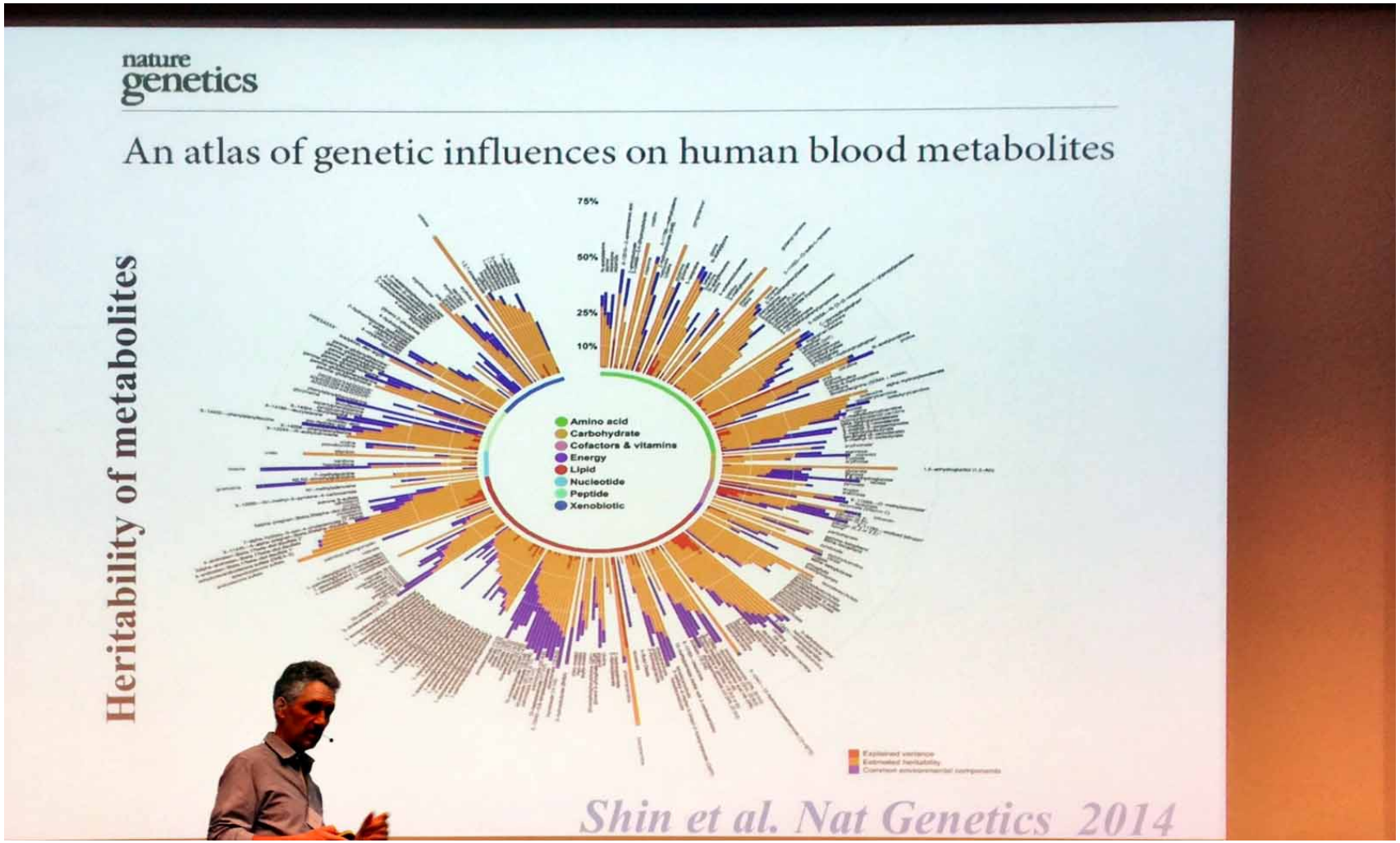


Equal

GlycanAge = TimeAge

Ludger

GlycanAge + Microbiome Diversity as an indicator for early warning of death in the elderly



Prof Tim Spector (KCL) talking about genes, glycans and microbes at the ION Ageing Matters Sympostium, Nov 2016

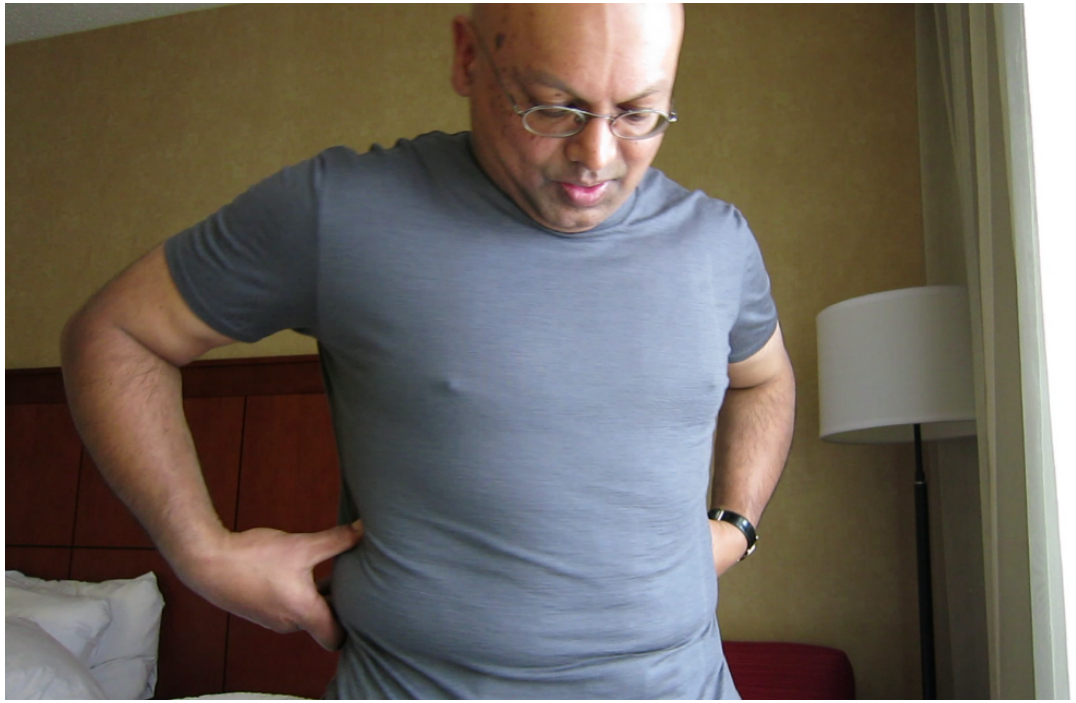

Ludger Fat Belly Project:
Quantified Self meets Lifestyle Medicine

Dr Daryl L. Fernandes, Oxford, 1987

Photo of a very tubby, possibly pre-diabetic Daryl – looking forward to years of ill health and possibly early death

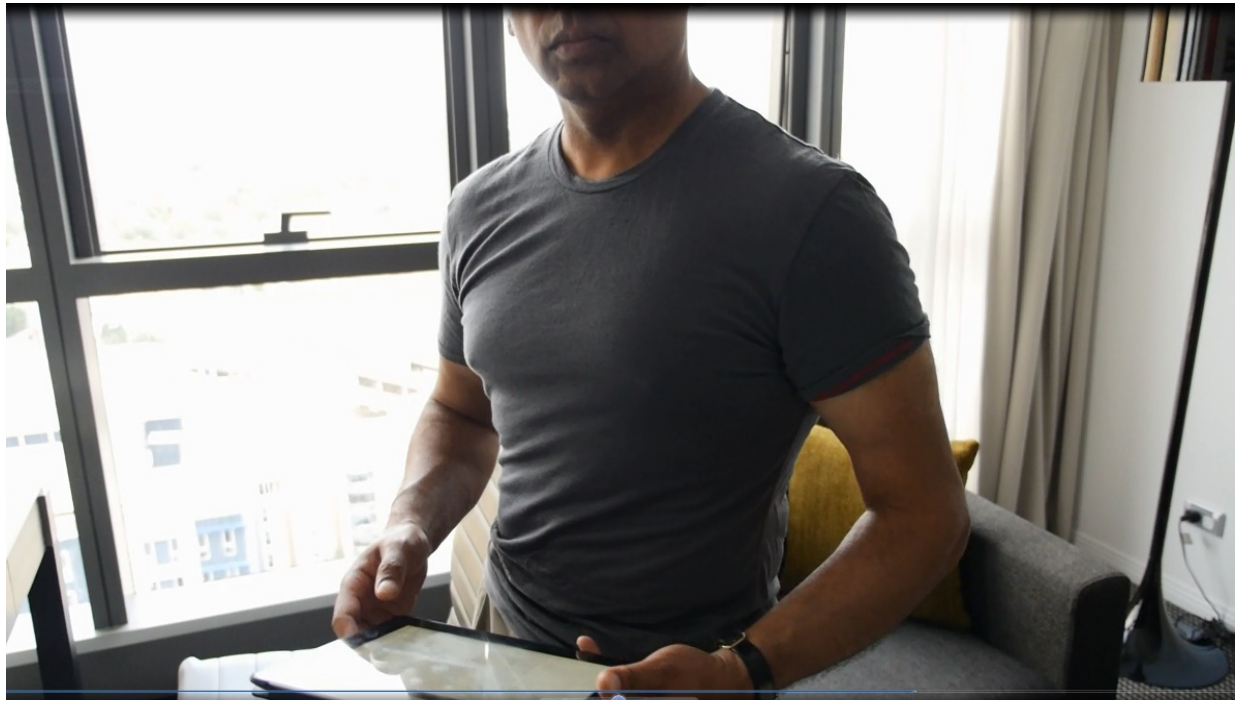
Avenna Fat Belly Project – Study in lifestyle medicine for chronic diseases

Feb 2013 – Got fat in USA



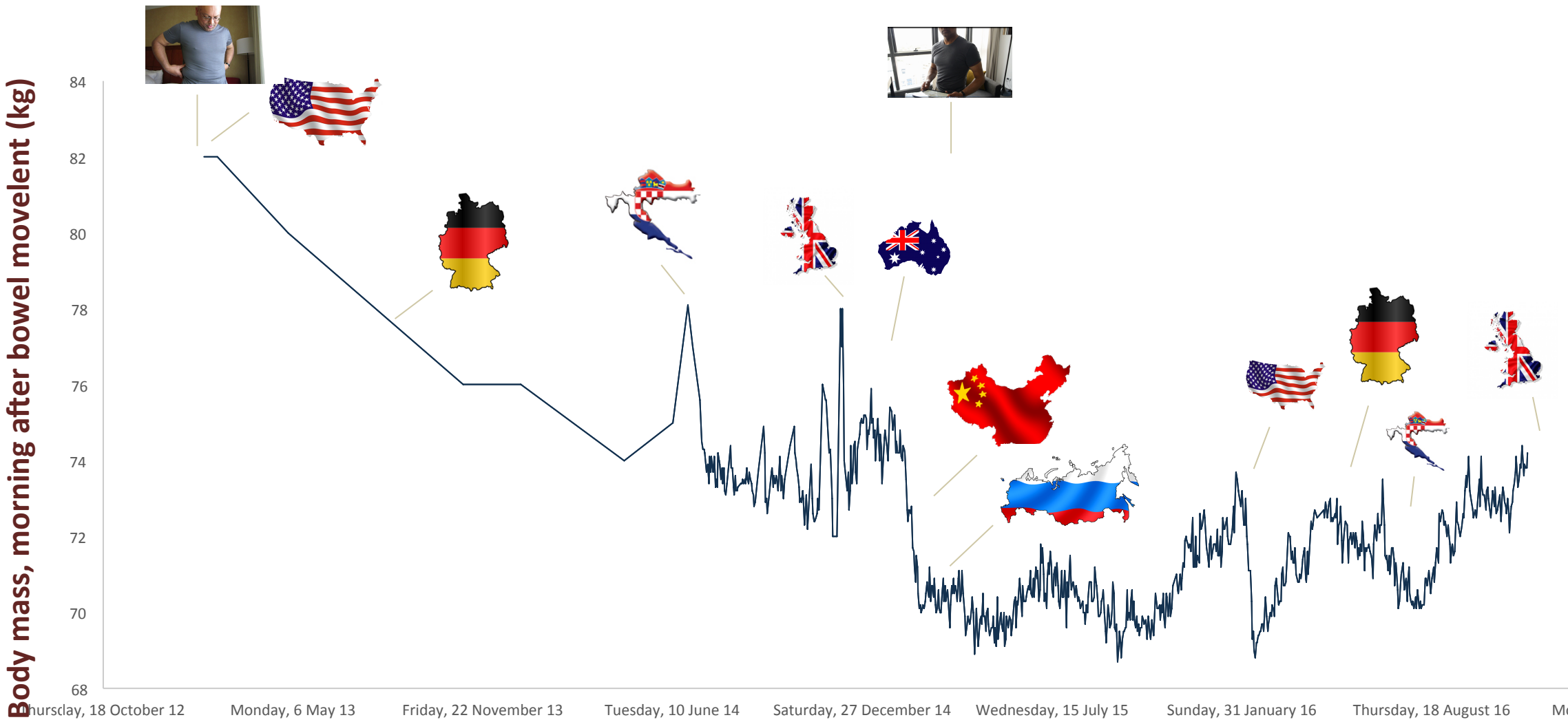
Ate more food and lost belly fat …

No starving, no gym!



Longitudinal N=1 Tubiness Experiments

How eating like a native affects my tubbiness



Careful food journalling

I’ve tracked everything I’ve eaten for the last three years

Bray Park, Fortitude Valley & Tyalgum
12 Feb – 4 Mar 2015 · New South Wales & Queensland

Orchard, Holland Village & Changi
4 – 7 Mar 2015 · Singapore

Shanghai - Huangpu, Pudong & Minhang
8 – 18 Mar 2015 · Shanghai

Gaining knowledge on how people get fat

From N= 1 to N=millions

N=1

N=10

N=100

N=1000

$N=10^4$

$N=10^5$

$N=10^6$

$N=10^7$

Study healthy/unhealthy individuals

Randomised Controlled Trials

Animal Studies: Lab, Natural Popns

Large scale prospective studies

NHANES, EPIC, Adventist, Healthy Shopper, Nurses,

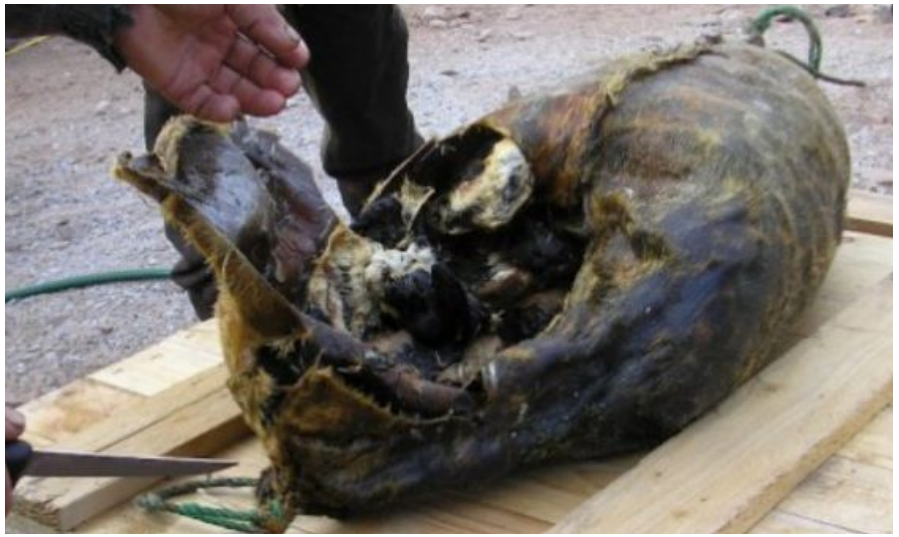
Population Studies (Longitudinal and Geographic)

China Study,
Philippines,
Blue Zones

Study science of natural and artificial diets

The Inuit: Healthy lifestyle or people at the limits of human survival?

The evidence is that Inuit live in brutal conditions and have suffered severe health problems for centuries



Lifestyle Medicine: What works for me

My Wizard of Oz moment

Food

Savour simple, tasty, colourful plant-based peasant food
Eat when hungry
Beware of hedonic hyperphagia

Movement

Walk a lot carrying heavy things
Use public transport
Wear barefoot shoes

Sleep

Sleep when tired
Get up when refreshed
Stop reading my ipad at night
(need to practice this more)


Quantified Ludger Programme:
Expansion of Fat Belly study to
aetiology of chronic diseases of aging

Glycomics + Quantified Ludger to Study Biomolecular Aetiology of Chronic Diseases

Glycosylation changes seem to be intimately linked to disease progression in many diseases

Mechanisms of disease: The human N-glycome☆

Gordan Lauc [a,b,*], Marija Pezer [b], Igor Rudan [c], Harry Campbell [c]

[a] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[b] Genos Glycoscience Research Laboratory, Zagreb, Croatia
[c] University of Edinburgh, School of Public Health Sciences, Edinburgh, Scotland, UK

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IgG glycosylation

ABSTRACT

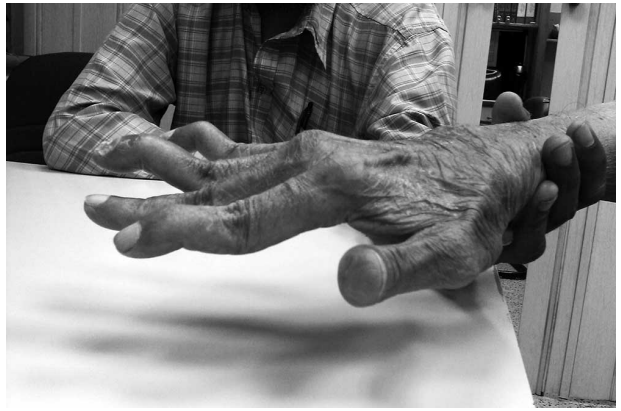
Background: The majority of human proteins are being modified by covalent attachment of complex oligosaccharides — glycans. Both glycans and polypeptide parts of a protein contribute to its structure and function, but contrary to polypeptide that is defined by the sequence of nucleotides in the corresponding gene, glycans are shaped by complex dynamic interactions between hundreds of enzymes, transcription factors, ion channels and other proteins.
Scope of review: An overview of current knowledge about the importance of N-glycans in normal human physiology and disease mechanisms, exemplified by IgG N-glycans.
Major conclusions: Recent technological development enabled systematic analysis of glycome composition in large epidemiological cohorts and clinical studies. However, the majority of these studies is still missing any glycomic component, and consequently also lacks this layer of biological information. Individual variation in glycosylation is potentially important for individualized disease risk, disease course and response to therapy. Evidence in support of this hypothesis is accumulating, but further studies are needed to enable understanding of the role of changes in protein glycosylation in disease.
General significance: Glycans are involved in virtually all physiological processes. Inter-individual variation in glycome composition is large, and these differences associate with disease risk, disease course and the response to therapy. This article is part of a Special Issue entitled "Glycans in personalised medicine" Guest Editor: Professor Gordan Lauc.

Quantified Ludger Glycomics signatures as biomarkers of health and disease

Collaboration between Ludger, Monopoli Lab and Clinical Partners

Clinical Partner: Patients

Inflammation, cancers, CVD, alcoholism, physical abuse, …



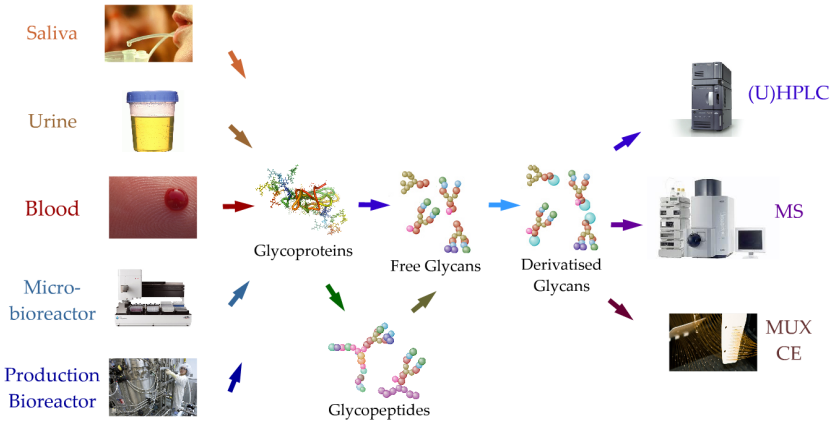
Monopoli Lab, RCSI Dublin:

Glycoprotein Fractionation

Fibrinogen, transferrin, alpha-1-acid-glycoprotein, …

Ludger: LongBow Glycomics

From Detailed LC-MS to HT GlycoPlates



Ludger Tree of Health Model for Precision Medicine

Nature + Nurture → Health or Disease

Symptoms of Health and Disease

Leaves and fruit

Body State

Tree trunk: Biochemistry, physiology

Glycome, Microbiome

Trunk of tree: reflects disease actuality

Nurture: Sun, Soil and Rain

Lifestyle – food, movement, sleep

Genome/Epigenome

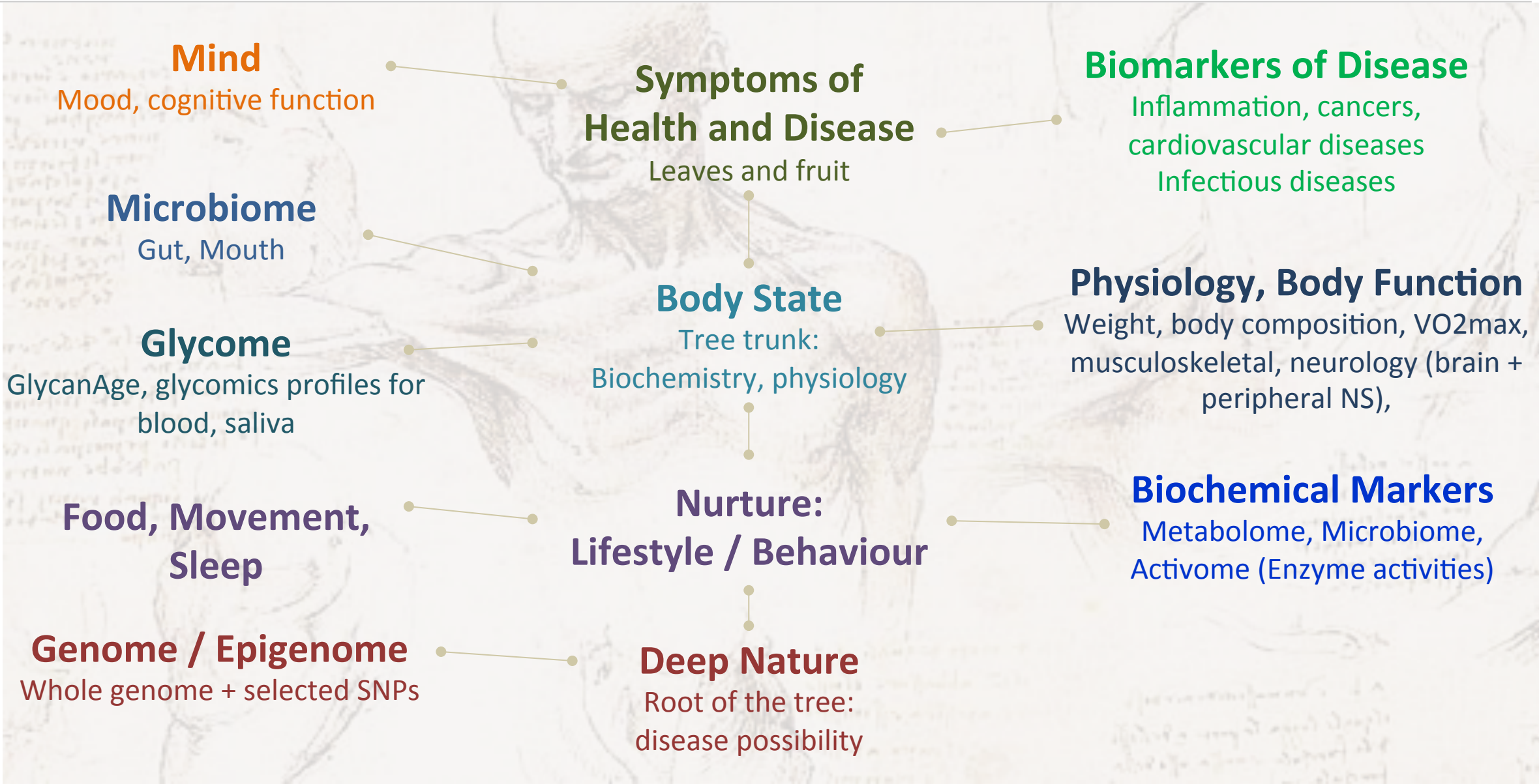
Root of the tree: disease possibility

Deep Nature

Tree of Life, Olympic National Park, Washington, USA

Quantified Ludger Programme – Longitudinal Studies for Precision Medicine

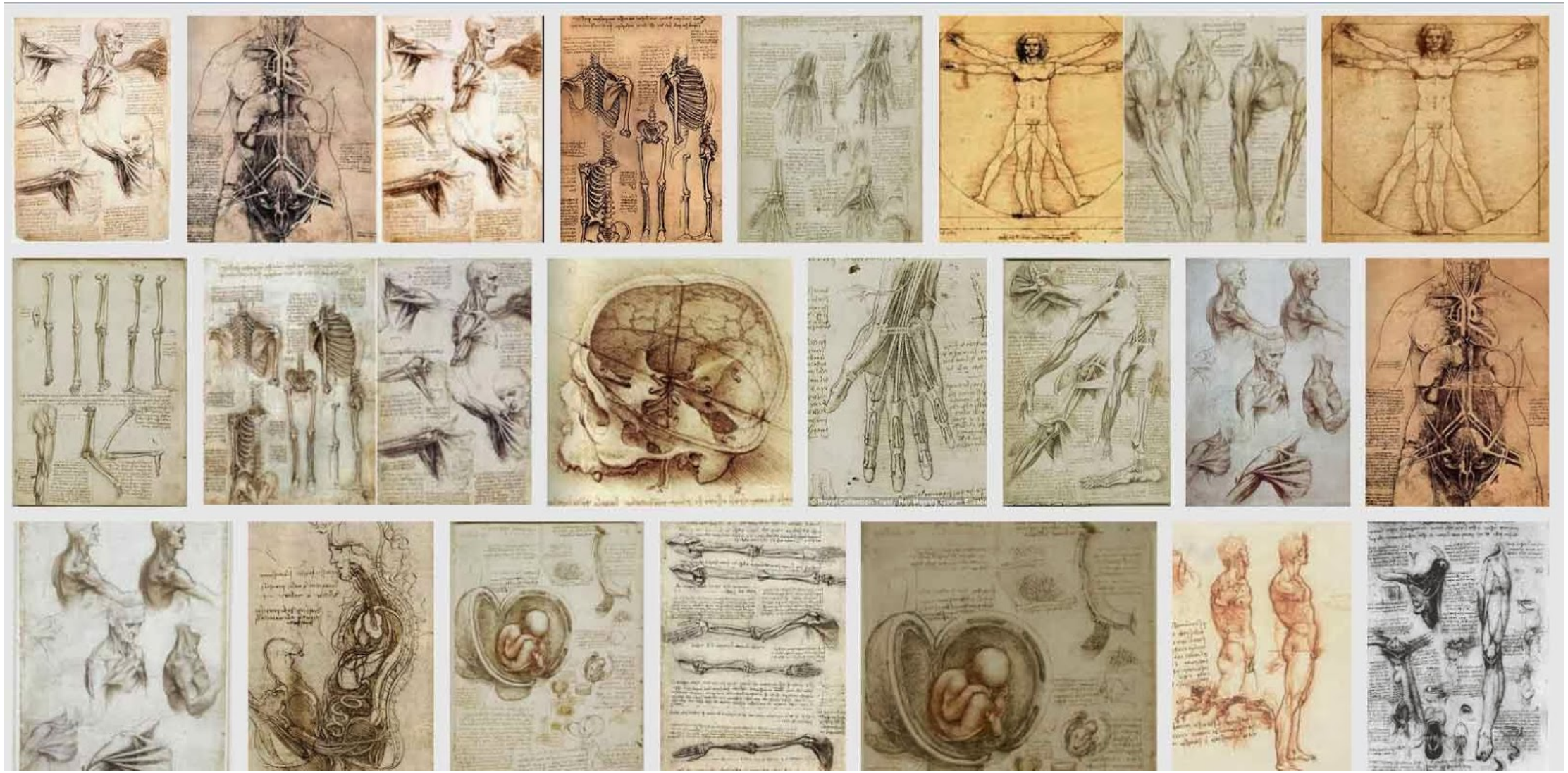
Expansion of Daryl's Fat Belly Studies – Detailed longitudinal studies of lifestyle, treatments and health biomarkers



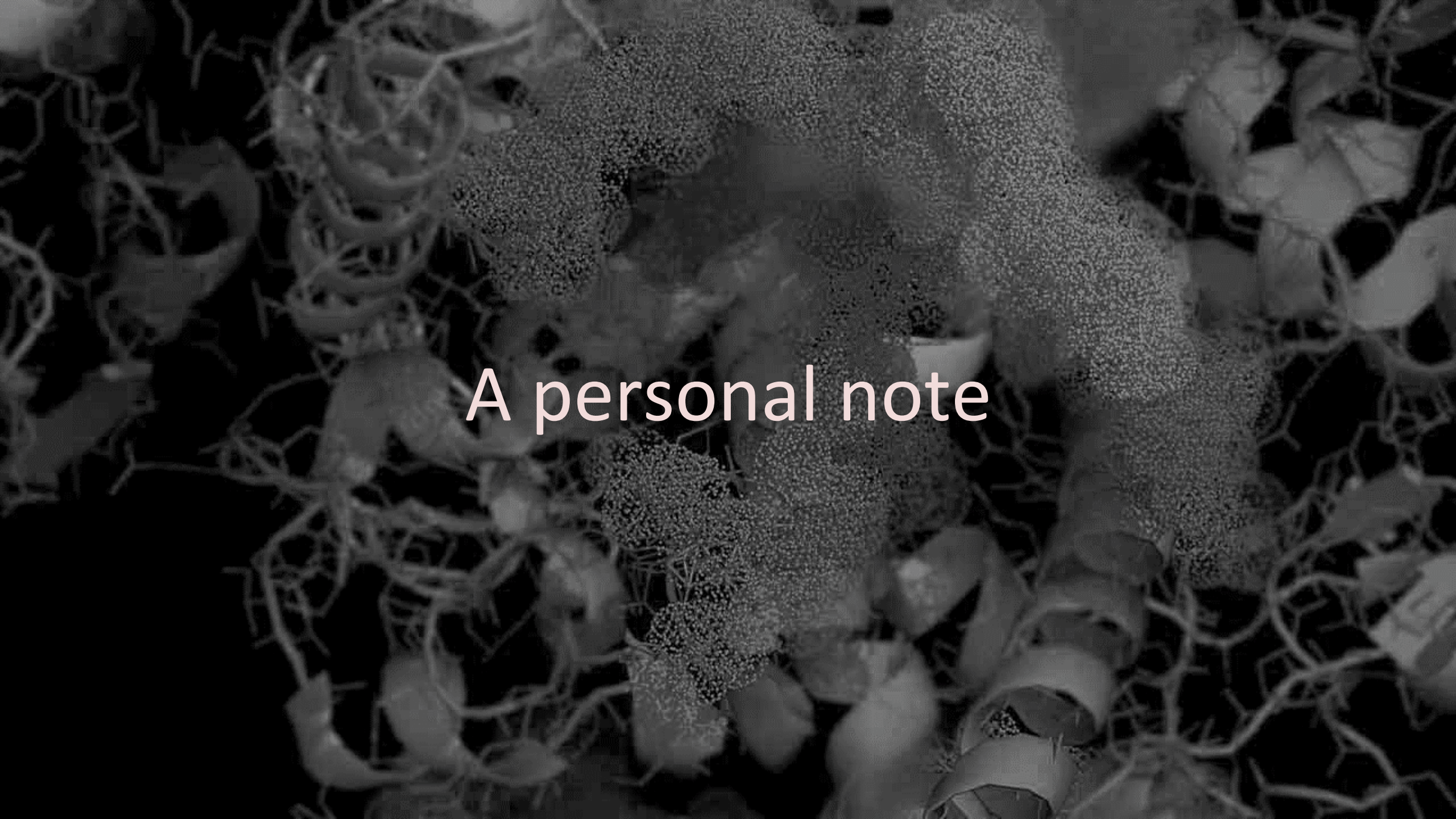
Ludger

Aims of Quantified Ludger: Develop System for Longitudinal Studies of Disease Aetiology

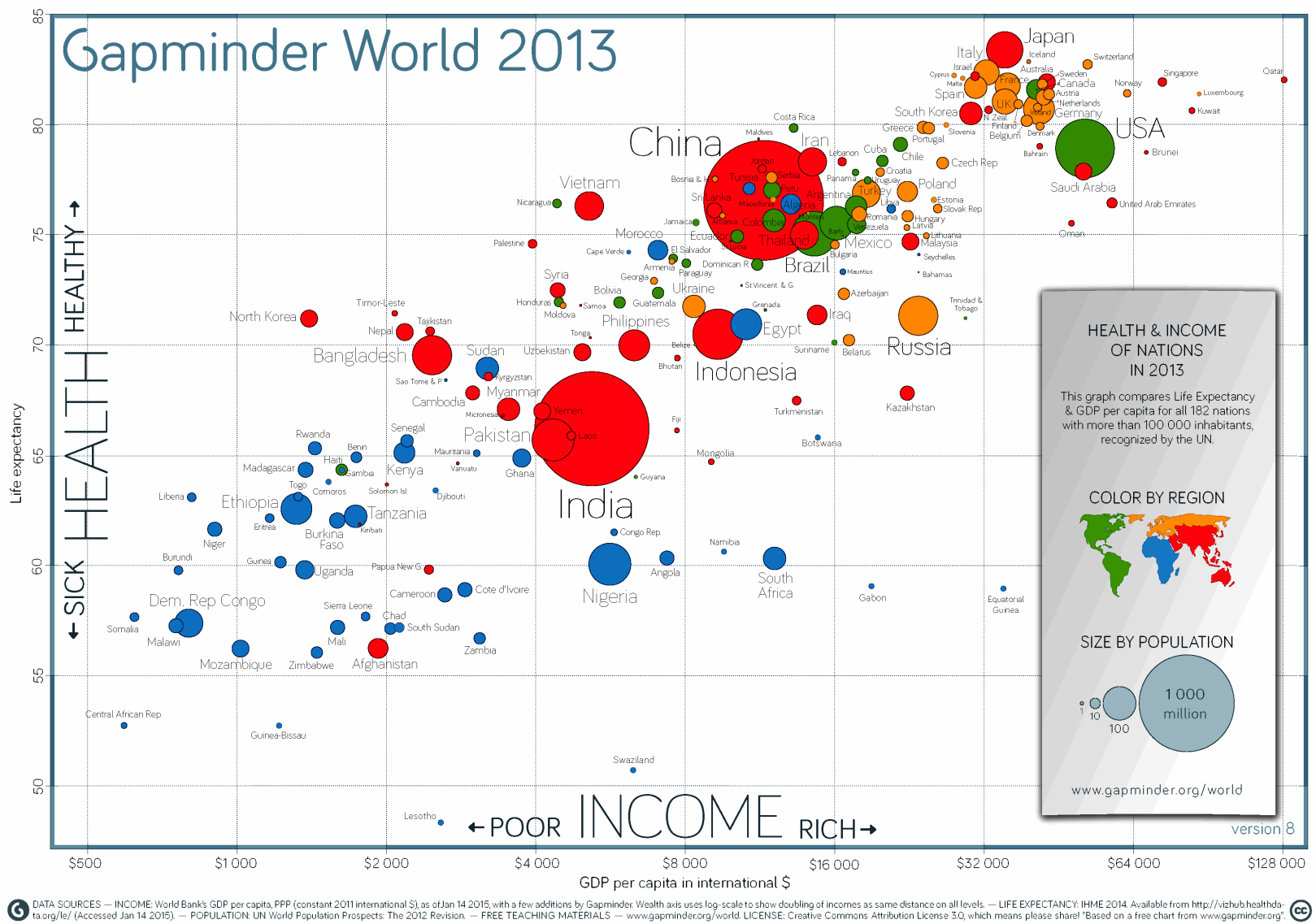
Nature + Nurture → Health or Disease: Focus on chronic inflammation, cancers and cardiovascular diseases



Leonardo da Vinci, Anatomy Drawings


A personal note

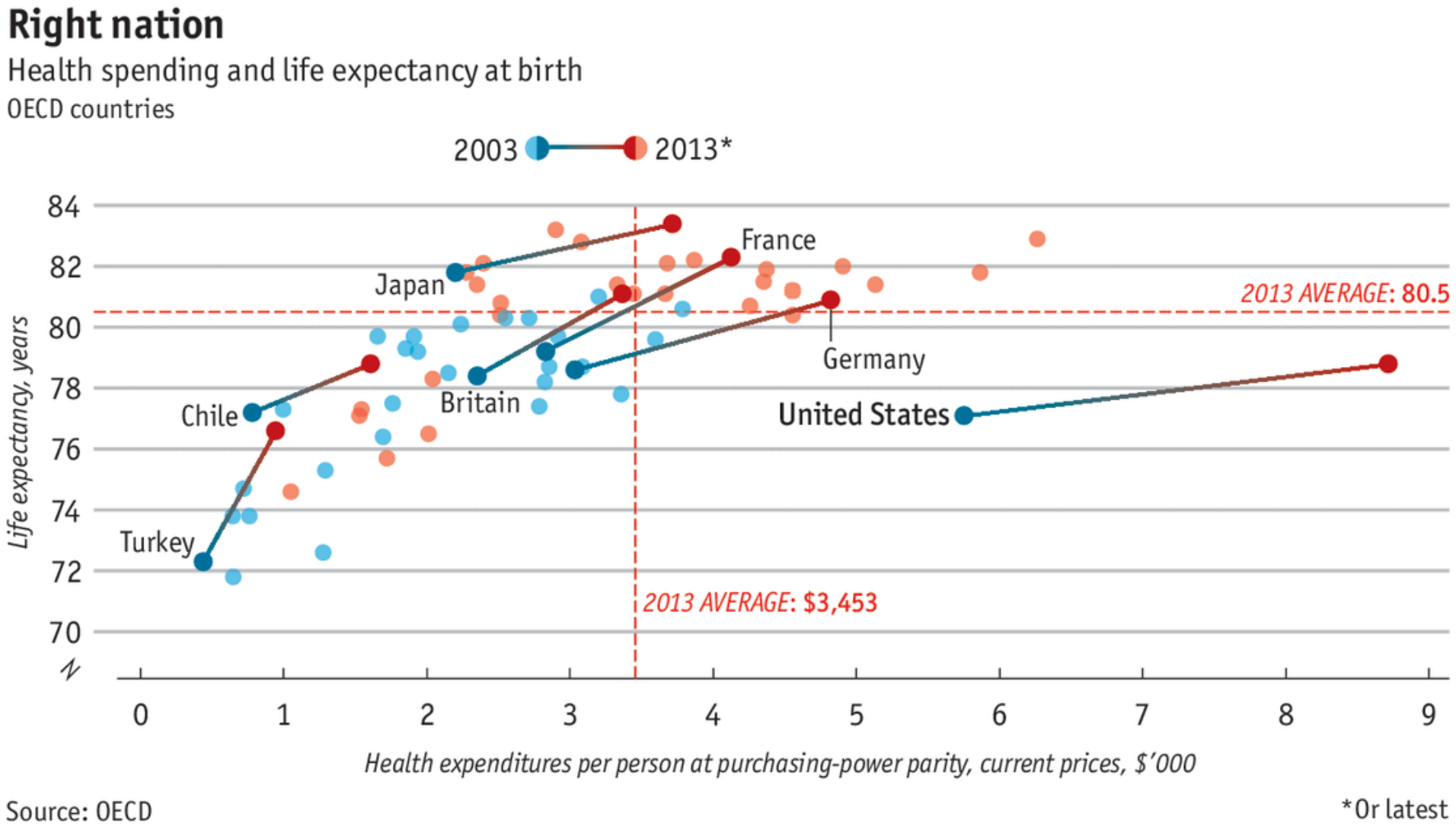
As the world develops, we are getting richer and living longer …



DATA SOURCES — INCOME: World Bank's GDP per capita, PPP (constant 2011 international $), as of Jan 14 2015, with a few additions by Gapminder. Wealth axis uses log-scale to show doubling of incomes as same distance on all levels. — LIFE EXPECTANCY: IHME 2014. Available from http://vizhub.healthdata.org/le/ (Accessed Jan 14 2015). — POPULATION: UN World Population Prospects: The 2012 Revision. — FREE TEACHING MATERIALS — www.gapminder.org/world. LICENSE: Creative Commons Attribution License 3.0, which means please share! "Based on a free chart from www.gapminder.org".

http://www.gapminder.org/posters/gapminder-world-2013//

… and the cost of healthcare is rising unsustainably



Reproduced from The Economist

… but are getting sicker …

‘Western’ industrialised lifestyles are a major driver for increased morbidity

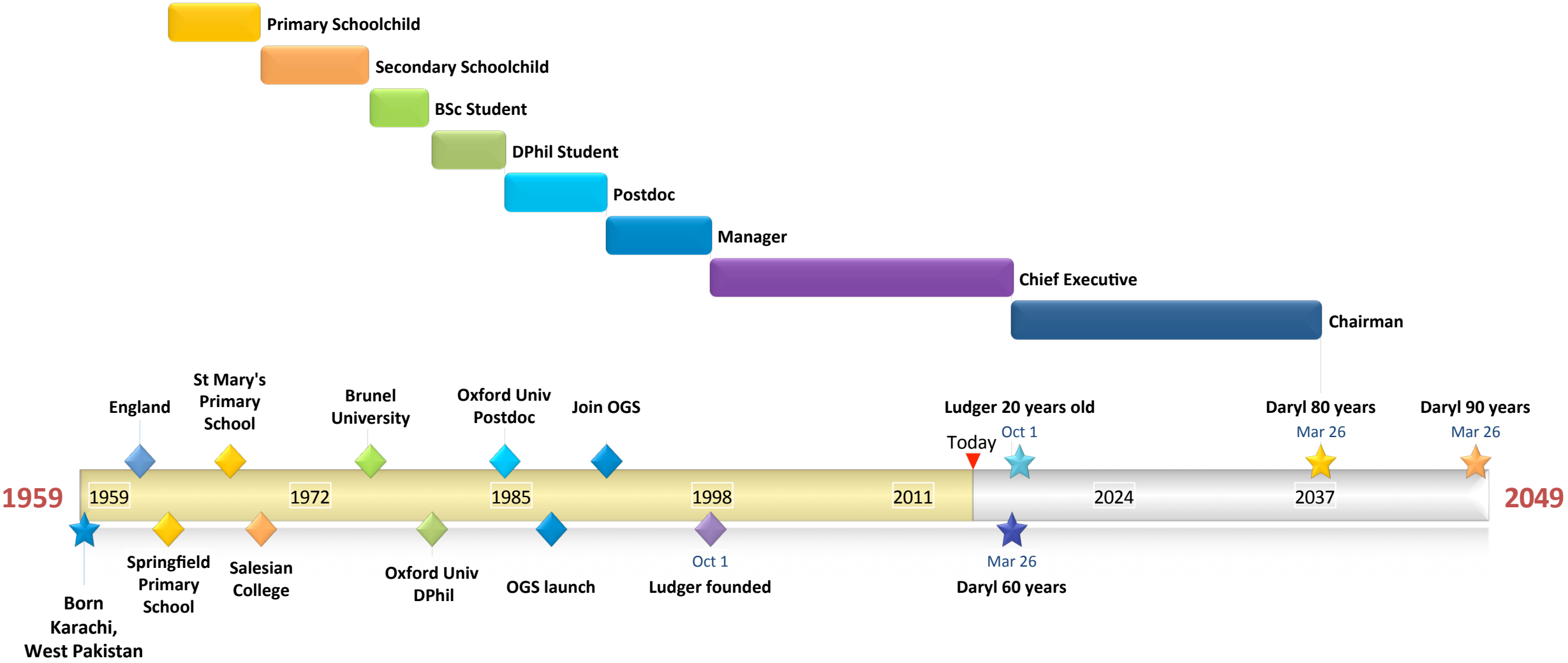
… we need better healthcare, medicines and health education

Mirror

Desperate father to sell kidney to fund treatment for his morbidly obese children

http://www.mirror.co.uk/news/world-news/desperate-father-sell-kidney-fund-5526985/

Daryl's Timeline for World Domination



Why I want to live a long, healthy life …

Ruben Fernandes, World Explorer






If you’re interested …

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Head of Business Development
claire.morgan@ludger.com