Systemic lupus erythematosus: pathogenesis, diagnosis, and treatment

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Summary and Discussion

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects a variety of organs and therefore includes a wide range of symptoms. SLE affects primarily women, with peak incidence in the reproductive years. Because the first symptoms of SLE usually manifest at a relatively young age, and because SLE currently has no cure, developing an effective therapy—preferably with few adverse effects—is essential for increasing the likelihood of achieving long-term remission. In addition to establishing an accurate diagnosis of SLE, it is also necessary to determine if, how, and to what extent various organs are involved in the disease process in order to select an appropriate treatment strategy. Further insight into the pathogenesis of SLE may provide novel targets for new therapeutic approaches.

Diagnosis

All current guidelines for managing SLE recommend performing a renal biopsy when renal involvement is suspected, as clinical and laboratory parameters are not sufficient for accurately assessing the histologic class of lupus nephritis (LN). As discussed below, the class of LN, which is determined by renal biopsy, guides the choice of treatment. The class of LN is determined primarily by the glomerular lesions present in the biopsy and is described in the current classification system for LN, which was published in 2004.[1 2]

In **Chapter 2** of this thesis, we report the results of our study of interobserver agreement with respect to the histopathologic lesions in class III and class IV LN. We focused on these two classes because these classes of LN present with the most severe renal involvement and are typically treated with aggressive immunosuppressive therapy. We took images of glomeruli reflecting the range of lesions that can be encountered in LN, and we distributed these pictures to the members of the Renal Pathology Society. We then asked participating nephropathologists whether glomerular lesions were present that would categorize the biopsy as class III/class IV. Our analysis revealed poor agreement among nephropathologists in terms of recognizing class III/class IV lesions. Importantly, the more experienced nephropathologists had a higher level of agreement for all lesions investigated, suggesting improvement can be made by training of pathologists. Other factors may also have influenced interobserver disagreement, including ambiguous definitions and non-adherence to classification methodology. The most ambiguous definition in the 2004 classification guidelines is the definition of “endocapillary proliferation”. Poor interobserver agreement was also observed with respect to assigning the distribution of glomerular lesions as either segmental (S) or global (G). Current guidelines and definitions on this

subject are both incomplete and inconsistent, possibly explaining the poor agreement among nephropathologists. The relevance of subdividing class IV LN into class IV-S and IV-G is the subject of ongoing debate. Haring *et al*. [3] performed a meta-analysis and found no difference in clinical outcome between patients with class IV-S LN and patients with class IV-G LN; nevertheless, some researchers argue that class IV-S LN and IV-G LN represent two distinct biological entities and should therefore remain separate in the classification.[4] Lastly, many of the respondents in our study did not appear to adhere to the definition of extracapillary proliferation, which requires involvement of at least one quarter of the glomerular capsular circumference.

These observations led us to re-evaluate the current classification of glomerulonephritis in SLE. In **Chapter 3**, we critically discuss all aspects of the current classification system, and we make suggestions for steps to improve the system. We also summarize the history of the classification system in order to provide insight into how the system evolved into its current form. In the current classification system, the is a lack of guidelines regarding how to approach certain aspects (for example, small or incomplete glomeruli), how to apply the classification system when evaluating multiple levels, and how to score extraglomerular lesions. Furthermore, the cutoffs separating class II from class I or class III are ambiguous. Our suggestions for improvement are based partly on expert opinion, partly on currently available new evidence, and partly on the future acquisition of new evidence. To improve the current classification system further, the goals of a classification system in general should be kept in mind.[5] Specifically, the classification system should: *i*) improve the quality of communication both between and among renal pathologists and clinical nephrologists; *ii*) provide a logical structure for categorizing groups of patients for epidemiological, prognostic (outcome), or intervention studies (*i.e.*, clinical trials); and *iii*) assist in the clinical management of individual patients in terms of therapeutic decision-making and prognostication. With respect to the first goal, clear and unambiguous definitions and guidelines should be provided; clear definitions may also improve interobserver agreement. With respect to the third goal, the current classification system certainly helps facilitate clinical decision-making. However, improvements can be made with respect to prognostication, particularly within class III and class IV LN. In order to achieve this, more evidence regarding the prognostic effects of individual histologic lesions such as fibrinoid necrosis is needed. For class III/IV LN, nearly all patients are treated with immunosuppressive therapy; therefore, it is not currently possible to study the natural course of individual histologic lesions in relation to outcome. However, one can study which lesions respond to therapy—perhaps even to a

specific therapy—and which lesions do not respond to therapy. For this purpose, repeat biopsies—although usually not available—would be extremely useful. Ideally, studies that relate histologic lesions to clinical outcome should be conducted in a group of patients who are treated using a similar protocol. Such studies may also help achieve a more evidence-based system for classifying LN.

Treatment

LN is one of the most severe manifestations of SLE and occurs in 20-60% of patients with SLE. To avoid end-stage renal disease and the resulting need for renal replacement therapy, LN must be treated both immediately and effectively. In **Chapter 4,** we compare, summarize, and discuss the current national and international guidelines for managing LN, which were published in 2012;[6-11] it is important to note that the principal statements were similar among all guidelines. With respect to class II LN, the focus of the therapeutic strategy should be on reducing proteinuria by inhibiting the renin-angiotensin-aldosterone system (RAAS). Moreover, some guidelines recommend the use of additional immunosuppressive medication in cases with high levels of proteinuria. To achieve remission in patients with class III or class IV LN, induction treatment should consist of intravenous cyclophosphamide (ivCYC) or mycophenolate mofetil (MMF) in combination with oral glucocorticoids, either with or without three pulses of intravenous methylprednisolone at the start of induction treatment. The optimal dosages of ivCYC and oral glucocorticoids, however, are less clear. Some guidelines base their recommendations on disease severity (*e.g.*, the presence of crescents in the renal biopsy), race (Caucasian or non-Caucasian), or the specific drug combinations used. Some guidelines also explicitly state that only patients with “active” lesions visible on renal biopsy should be treated. Although this may seem obvious, it should nevertheless be explicitly discussed between the nephrologist and nephropathologist. All guidelines recommend including either MMF or azathioprine (AZA) in the maintenance phase of treatment, although some guidelines prefer MMF over AZA.

For the treatment of class V LN less robust evidence is available, which is reflected in the recommendations. Although most guidelines recommend RAAS inhibitors with the addition of immunosuppressive medication in case of nephrotic-range proteinuria, one guideline advises immunosuppressive medication irrespective of the level of proteinuria. Furthermore, which immunosuppressive medication is preferred—if any—is unclear. As adjunct therapy to the specific strategies outlined above, controlling blood pressure, treating hyperlipidemia

with statins, and treating proteinuria with RAAS inhibitors are recommended. In addition, hydroxychloroquine is recommended for all SLE patients, despite a lack of randomized controlled trials to support its use in LN. Despite the lack of clinical trial-based evidence for treating refractory LN, the guidelines generally recommend switching from MMF to ivCYC—or from ivCYC to MMF, if appropriate—if induction treatment fails. If this strategy fails, one of the recommendations is the use of rituximab, a humanized antibody directed against the B cell antigen CD20. However, given that the LUNAR trial, which included rituximab as an addition to steroid-MMF combination therapy, failed to reach the study endpoint, the efficacy of rituximab in this context has not yet been demonstrated in a randomized clinical trial.[12]

Designing a successful randomized clinical trial with SLE patients poses many challenges. First, selecting the study population can be difficult, particularly given the extremely heterogeneous disease manifestations among patients. Even though LN is only one such disease manifestation, patients with LN are a heterogeneous population with respect to renal involvement. Second, the disease manifestations, disease severity, and response to treatment differ between races, further increasing the clinical heterogeneity of the study population. Selection of the treatment and control regimens is also a key factor when designing a trial. The control regimen should leave room for measurable and meaningful improvement. Finally, selecting appropriate response criteria is essential to the outcome of a trial. However, as reflected by the differences in response criteria among the guidelines discussed above, no consensus has been reached with respect to what these criteria should be. Measures of irreversible damage (for example, the extent of chronic changes observed on renal biopsy) may be utilized to either stratify patients or balance randomization at baseline. These measurements can also be incorporated in the endpoint analyses to ensure that treatment- and/or disease-related deterioration—which can be overlooked when scoring disease activity alone—has not occurred.[13 14] Performing a post-treatment renal biopsy may also provide additional insight into which histologic lesions respond to therapy and which lesions do not. Evidence also suggests that gene expression profiles may in the future be used to predict which patients will likely respond to therapy and which patients will likely not respond.[15] Given the high heterogeneity of SLE patients, developing patient-tailored treatments is essential, but will be extremely difficult to achieve. Therefore, large, collaborative studies that involve all relevant medical disciplines are needed.

Pathogenesis

To investigate the pathogenesis of SLE and LN, we focused on DNA. First, we studied microchimerism (Mc), which is the presence of a small number of genetically distinct cells (of any type and originating from a different zygote) in an individual. Fetal Mc arises from fetal cells that enter the maternal circulation. We used differences in genetic polymorphisms between individuals to detect Mc. Second, we studied the contribution of known lupus susceptibility polymorphisms in familial lupus nephritis. Both of these approaches are discussed below.

SLE

Mc has been implicated in the pathogenesis of SLE (for review, see Kremer Hovinga *et al*.[16]). Although the precise role of Mc in SLE is unclear, three hypotheses have been suggested: *i*) the chimeric cells induce a graft-versus-host response; *ii*) the chimeric cells induce a host-versus-graft response; and *iii*) chimeric cells play a beneficial role in repair mechanisms. Further studies regarding the role of Mc in SLE are described in **Chapter 5** and **Chapter 6**. In **Chapter 5,** we report the occurrence and number of chimeric cells in the peripheral blood of SLE patients and control subjects. Our analysis revealed that SLE patients have a significantly higher prevalence of Mc compared to control subjects (54.5% versus 12.6%, respectively; P=0.03). Furthermore, when analyzing only patients and control subjects with Mc, the median number of fetal chimeric cells was significantly higher in SLE patients compared to control subjects (with 5 and 2.5 chimeric cells per 10^6 cells, respectively; P=0.046).

In previous studies, the detection of Mc was limited to the detection of male Mc (by identifying the Y chromosome). Here, using insertion-deletion polymorphisms and null alleles, in addition to the Y-chromosome, we were able to detect and distinguish Mc from different sources. We found that when present, Mc was usually fetal in origin in both patients and control subjects. Strikingly, we also found that in SLE patients with Mc, the chimeric cells originated from several relatives in 50% of cases; in contrast, in control subjects with Mc, the chimeric cells originated from only one relative in 100% of cases. We found no correlation between Mc and either clinical or laboratory parameters related to SLE. Because the transfer of fetal chimeric cells occurs during pregnancy (when the mother is exposed to the fetus), we reasoned that the higher prevalence of Mc in SLE patients occurred either because SLE patients acquire more fetal cells than control subjects during pregnancy, or because Mc is cleared to a lesser extent in SLE patients. To test these two

possibilities, we compared pregnant SLE patients with healthy pregnant control subjects (**Chapter 6**). We measured the level of Mc in the peripheral blood of pregnant women at 30 weeks of gestation, just after delivery, and 1 week, 6 weeks, 3 months, and 6 months after delivery. Compared to control subjects, SLE patients had a significantly higher number of fetal chimeric cells in the granulocyte fraction just after delivery; no difference was observed at any other time point measured. Importantly, at both 3 and 6 months after delivery, no fetal chimeric cells were detected in either SLE patients or control subjects. This finding is in contrast to the Mc detected in both patients and control subjects many years after their last pregnancy (as described in **Chapter 5**), shedding new light on the dynamics of fetal Mc. This finding also argues against our notion that the increased prevalence of Mc among patients with SLE years after their last pregnancy is due to the acquisition of more chimeric cells during pregnancy or reduced clearance of chimeric cells after pregnancy. Rather, it suggests that chimeric cells are cleared from the peripheral blood rapidly after pregnancy and then reappear years later, possibly originating from non-circulating fetal chimeric stem cells. Although the trigger for the reappearance of chimeric cells in the peripheral blood is unknown, it may be related to disease activity and/or tissue damage.

With respect to Mc in the peripheral blood mononuclear cell fraction, we found no difference between patients and control subjects at any time points examined. The role of fetal chimeric cells in the granulocyte fraction in SLE remains unclear. One possibility is that the chimeric neutrophils may undergo NETosis (the formation of neutrophil extracellular traps, or NETs), leading to the presentation of chromatin to the immune system. This “chimeric NETosis” may be more immunogenic than “self NETosis”. Nevertheless, it should be noted that the patients in this study were already diagnosed with SLE, rather than being in a preclinical phase of the disease. Therefore, this increase in Mc may be either a consequence or cause of the disease—or possibly both. Regarding the role of Mc in SLE in general, Kremer Hovinga *et al*. proposed three hypotheses, two in which Mc plays a pathogenic role and one in which increased Mc is a side effect of SLE. This putative side effect could be the result of repair following damage, or it could be the result of an altered immune system (either intrinsic or iatrogenic in nature). However, none of the aforementioned hypotheses stand out in terms of supportive evidence obtained to date. Thus, the chimeric cells could be beneficial, detrimental, or even inconsequential to the host. To determine whether Mc is a cause or consequence of SLE, it would be interesting to test whether SLE patients have more fetal chimeric cells than healthy control subjects *before* their first symptoms occur. Unfortunately, however, this would require repeated blood draws from a large number of healthy women

over a prolonged period of time, which is simply not feasible. To gain further insight into the role of Mc in SLE, it would also be interesting to determine the precise identity (*i.e.*, cell type) of the chimeric peripheral blood mononuclear cells. Furthermore, to determine whether chimeric granulocytes undergo NETosis, an animal model could be developed in which the chimeric cells are labeled (for example, with GFP). Moreover, the hypothesis that the increased prevalence of Mc in SLE is due to damage repair during SLE disease activity could be tested by following subjects over time, collecting clinical data, and then correlating these data with sequential data regarding Mc in the same patients. This approach could be performed in SLE patients and/or an animal model. If the results indicate that chimeric cells play a role in initiating and/or maintaining SLE, these chimeric cells could then be targeted (for example, using anti-HLA antibodies) and removed from the patient, providing a strategy for treating SLE in these patients.

The role of Mc in disease can also be examined from beyond the field of SLE, as the prevalence of Mc is also increased in several other autoimmune diseases.[17-19] This suggests that these autoimmune diseases have a common pathogenic basis. Alternatively, the increased prevalence of Mc could be a bystander effect. These diseases manifest as a chronic state of inflammation, which could facilitate the recruitment of chimeric stem cells; alternatively, the tissue damage caused by these diseases could lead to repair by chimeric cells (among other cells). In some cancers, chimeric cells are believed to play a beneficial role (for review, see Fugazzola *et al*.[20]) For example, chimeric cells may be involved in the immune surveillance of cancer cells, thereby providing a protective effect. Increased Mc in tumor tissue compared to adjacent benign tissue supports the notion of the recruitment of chimeric cells for tissue repair. If Mc plays a similar role in diseases in general—including various autoimmune diseases, inflammation, and cancer—the most likely role of chimeric cells is to repair damaged tissue. The involvement of chimeric cells in tissue repair may be beneficial to the host, or it may be an "innocent bystander" effect.

Lupus nephritis

Genetic factors are believed to play a significant role in the etiology of SLE. In **Chapter 7**, we compare and contrast familial and sporadic forms of lupus nephritis with respect to clinical parameters, serology, histologic class, the activity and chronicity indices (AI and CI), the number of glomerular monocytes/macrophages, and the contribution of known lupus susceptibility polymorphisms. We found that the frequency of juvenile onset was higher among familial LN patients compared to sporadic LN patients (50% versus 22%, respectively;

P=0.03). In addition, 44% of familial LN patients were male, compared to 12% of sporadic LN patients (*P*=0.004), and familial LN patients had a higher likelihood of progressing to advanced renal disease (25% versus 7% for sporadic LN; *P*=0.03). However, we found no difference in any of the histologic parameters explaining the observed difference in renal outcome between familial LN and sporadic LN. To provide a composite measure of genetic susceptibility, we calculated a genetic risk score (GRS). Our analysis revealed that the GRS did not differ significantly between familial LN patients and sporadic LN patients. Furthermore, in families in which LN clusters, the GRS was similar between each proband and the proband's unaffected relatives, providing further evidence that an accumulation of susceptibility alleles likely does not underlie familial LN. Therefore, the underlying differences between familial LN and sporadic LN remain unknown. Future experiments could include whole-exome sequencing in families with several affected members, which may identify rare genetic variants.

It's all a matter of perception

Perception can be defined in several ways, including *i*) the ability to see, hear, or become aware of something through the senses and *ii*) the way something is regarded, understood,

Figure 1. "My wife and my mother-in-law"
British cartoonist William Ely Hill (1887–1962) published "My Wife and My Mother-in-Law" in *Puck*, an American humor magazine, on 6 November 1915, with the caption "They are both in this picture—Find them" (panel A). However, the oldest known form of this image is an anonymous 1888 German postcard (panel B).

or interpreted. The second definition applies to the classic image "My wife and my mother-in-law" by W.E. Hill (Figure 1), and both definitions pertain to many aspects of this thesis.
Perception plays a major role in diagnosing SLE in general and LN in particular. Because SLE can present clinically with many "faces", combining the right perception of symptoms with other parameters often leads to the eventual diagnosis of SLE. In 2012, a new classification system for use in diagnosing SLE was proposed.[21] One remarkable change was the addition of the criterion that a diagnosis of SLE can be established based on the presence of LN in a renal biopsy combined with the presence of circulating anti-nuclear antibodies (positive ANA test). This criterion—combined with the principal role of a renal biopsy in guiding the treatment of LN—puts additional emphasis on the way in which the pathologist perceives the biopsy results. When evaluating a renal biopsy, both definitions of perception apply. First, all sections, special stains and immunofluorescence must be evaluated carefully in order to obtain a correct diagnosis and classification. Even the presence of focal "proliferative" lesions in only one or a few glomeruli will determine the treatment strategy in an individual patient. Second, interpretation also plays a major role in classifying a biopsy. Even if a new classification system is proposed by a panel of experts, if that classification system—including all of its definitions—is not interpreted by the users as intended by its creators, the system may be useless. Difficulties arising from one or both types of perception can lead to low interobserver agreement. One possible solution is to train pathologists in order to improve their ability to "see". This approach—along with clear, practical, uniform, and careful formulation of definitions in the classification system—may also affect their understanding and/or interpretation of the classification system. This is not an easy task, as most experienced

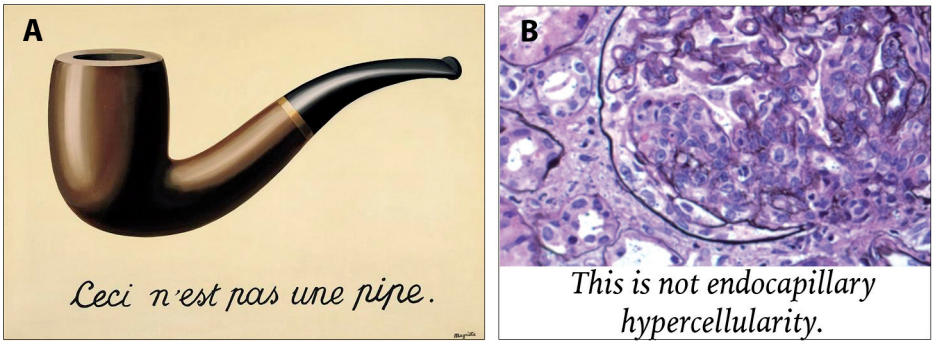

Figure 2. The treachery of images: This is not a pipe
Panel A shows the 1929 painting entitled "*Ceci n'est pas une pipe*" ("This is not a pipe") by René Magritte. Panel B shows an image depicting endocapillary hypercellularity.

pathologists have a preconceived mental image of what they perceive as *e.g.* endocapillary hypercellularity. Can words replace what the pathologist sees in a picture? And which has more authority, the picture or the words? This struggle is represented in the 1929 painting entitled "*Ceci n'est pas une pipe*" ("This is not a pipe"; this painting is commonly referred to as "The Treachery of Images") by René Magritte (Figure 2A). The paragon of complete agreement may only be achieved if the pathologist is replaced by a computer. Although replacing pathologists with computers is not likely to occur in the near future, computer-aided diagnostic technologies (such as automated screening of Pap smear results) are being developed. In breast cancer, a computer model based on a plethora of microscopic features in tissue microarray samples, as analyzed by the computer, was able to predict patient survival more accurately than conventional histologic parameters (*e.g.*, tumor grade).[22] In the future, automated analysis of renal biopsy images may help pathologists obtain a more accurate, more reliable, and more reproducible assessment of specific prognostic features. Alternatively—and analogous to the breast cancer study discussed above—computer models may be able to perceive features relevant to prognosis that are not currently identified by performing a conventional examination.

In clinical trials, the perception and documentation of treatment effects are essential to the development of new treatment strategies. However, determining treatment effect is often hindered by several factors, including the way in which the resulting change in symptoms is both perceived and defined. With respect to lupus, one of the major challenges lies in finding equally effective—or more effective—drugs with fewer and/or less severe side effects. For example, cyclophosphamide, although often administered for a limited period of time, can have severe side effects, including reduced fertility. Although mycophenolate mofetil (MMF) does not have these fertility-related side effects, it does have other side effects, including an increased risk of severe infections. Furthermore, oral glucocorticoids have been the standard treatment for many decades. Despite the existence of steroid-sparing treatment strategies, many SLE patients are treated with long-term courses of oral glucocorticoids, which can have long-lasting side effects, including suppression of the hypothalamic-pituitary-adrenal axis, Cushingoid appearance, hirsutism or virilism, impotence, menstrual irregularities, peptic ulcer disease, cataracts and/or increased intraocular pressure/glaucoma, myopathy, osteoporosis, and vertebral compression fractures. However, before oral glucocorticoids can be eliminated from the standard treatment regimen, new trials must be performed to compare steroid-free regimens with classic steroid-containing regimens. For example, a trial is currently underway (RITUXILUP NCT01773616) comparing the "standard" oral

glucocorticoid/MMF regimen with a regimen of induction therapy that includes two doses of rituximab and methylprednisolone followed by maintenance with MMF. This study also circumvents a problem commonly encountered with studies to test a new drug for LN: many drugs are tested either as an add-on or in refractory disease. In these settings, defining the primary endpoint is extremely important; specifically, it is important to address the following question: What do we *perceive* to be a clinically relevant and reasonable response? One may also wonder whether the clinical parameters that are currently used as the response criteria truly represent the actual disease activity and chronicity, and—consequently—whether protocol biopsies may be a valuable addition for determining renal response.

When studying the role of Mc in SLE, one must always keep in mind that more information might be found beyond the limits of our perception. Although Mc is often reported as a binary outcome (*i.e.*, either present or absent), this view is likely only one part of a much bigger picture. For example, an absence of Mc may indicate that the subject truly does not carry any chimeric cells, or it may mean that chimeric cells are present but are below the current detection limit (*i.e.*, fewer than 1 chimeric cell per 100,000 "host" cells); in other words, absence of proof is not proof of absence. This begs the question of whether the presence of cells that we cannot detect has any biological relevance. As stated by Elliot Eisner, "Not everything that matters can be measured, and not everything that is measured matters."[23] Because the number of chimeric cells in an individual is extremely low, isolating and characterizing these cells can be quite difficult. To determine the phenotype of these chimeric cells, many studies—including those presented in this thesis—use an indirect method in which Mc is detected in a specific subset of cells. Drabbels *et al.* used a method in which fluorescence-activated cell sorting was used to isolate chimeric cells based on HLA mismatch.[24] Some animal studies used a variation of this method by isolating fetal chimeric cells of GFP-positive offspring.[25] Although it is clearly preferable to study Mc in human subjects, animal studies currently offer the only platform for studying the dynamics of Mc, its effects, and factors that influence Mc.

In **Chapter 7**, we report that patients with familial LN are more likely to progress to advanced renal disease compared to patients with sporadic LN. However, none of the parameters investigated were sufficient to explain this perceived difference. For example, biopsies from familial LN patients revealed similar disease severity as biopsies from sporadic LN patients. We also found no difference between familial and sporadic cases with respect to their genetic risk scores, suggesting either that an accumulation of susceptibility alleles does not lead to familial LN, or that risk alleles other than the ones studied here play a role. In this

respect, exome sequencing may be a useful strategy for identifying rare genetic variants that may play a role in familial LN.

Concluding remarks

In daily practice, perception—which is defined both as the ability to see, hear, or become aware of something through the senses and as the way something is regarded, understood, or interpreted—is an essential tool for diagnosing and treating SLE in general and LN in particular. Moreover, research regarding the pathogenesis, diagnosis, and treatment of this disease hinges on how we observe the outcome and results, how we interpret those results, and what we perceive to be clinically relevant. In both clinical practice and research, we should always be aware of the strong influence of our *perception*.

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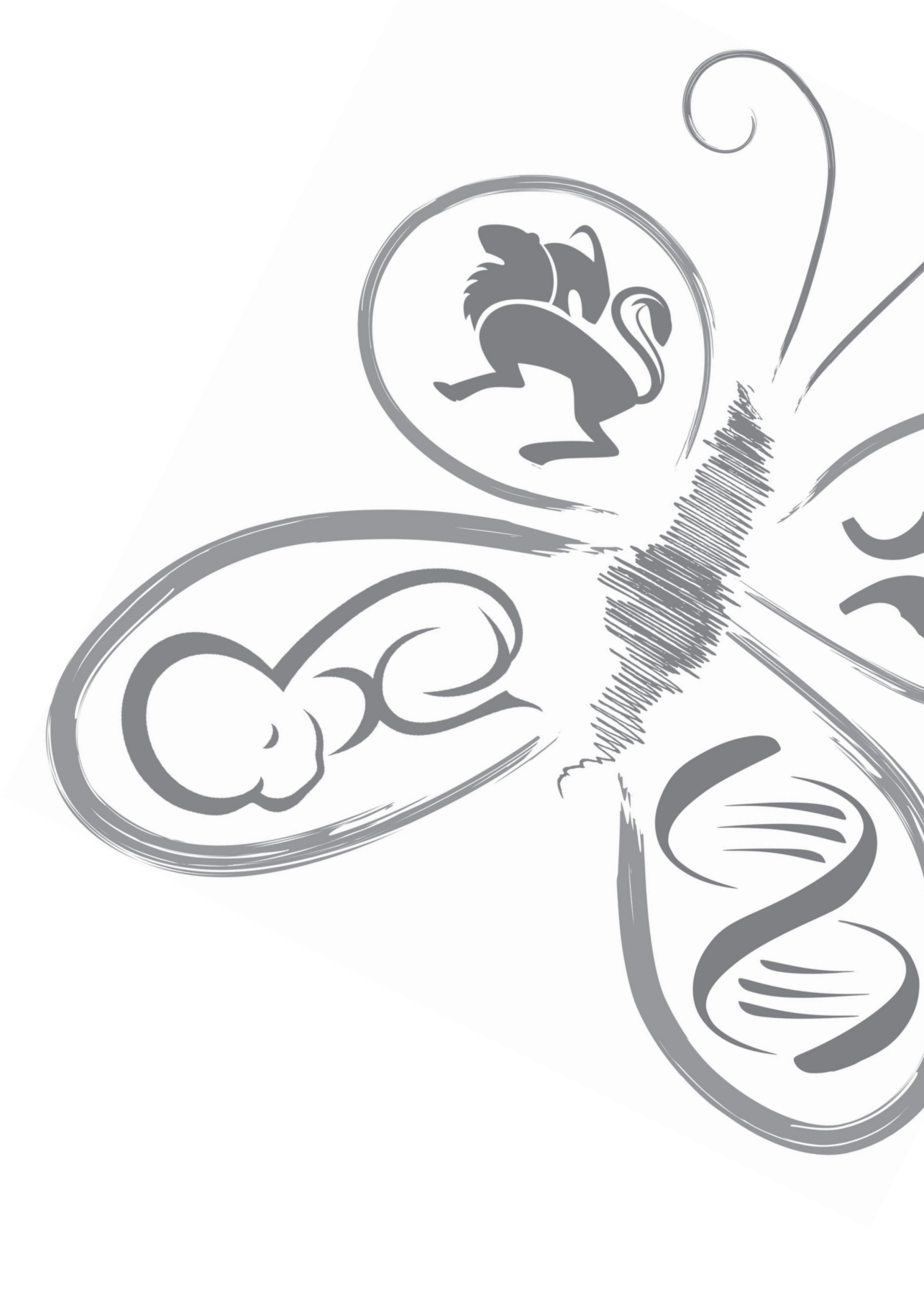

Addenda