In the late 19th century, Francis Galton, Charles Darwin’s cousin, first thought of using twins to investigate the role of heredity and environment in human life. Since then, twin studies in biology, medicine and psychology have had a considerable impact on the scientific community; this has generally been for the better although, in the case of ‘eugenic’ politics, it has also been for the worse. Galton realized that, because twin pairs (whether identical or non-identical), or differences between identical twins for a given disease, suggest that the disease is probably due to non-genetically determined factors.

In the century following Galton’s publication, additional methods were used to exploit the value of twins, such as comparing identical twins with and without a disease. Immunological investigations in immune-mediated disease are an outstanding example of the potential of twin studies. Autoimmune diseases affect up to 5% of the population and are a major cause of morbidity and mortality. The vast majority of twin studies in immune-mediated diseases have been carried out in autoimmune diseases, including insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis (AS) and coeliac disease. These diseases cover a spectrum of organ-specific and non-organ-specific diseases and will be the focus of this review.

In such diseases, the impact of genetic factors is so substantial, and the identity of all immune response genes sufficiently uncertain, that

Twins: mirrors of the immune system

Marco Salvetti, Giovanni Ristori, Roberto Bomprezzi, Paolo Pozzilli and R. David G. Leslie

Twin studies are a powerful tool to assess genetic and non-genetic factors in multifactorial, immune-mediated diseases. Here, Marco Salvetti and colleagues review important results from such studies and highlight their potential value. Future developments that should help to realize the potential of twin studies are discussed.

18 Lombardi, G. et al. Type-I interferon maintains the survival of anergic CD8+ T cells. J. Immunol. (in press)
21 Van Parp J. and Abbas, A.K. (1998) Non-identical twins probably reflect a role for genetic factors leading to the disease. By contrast, similar concordance rates for a disease in twin pairs (whether identical or non-identical), or differences between identical twins for a given disease, suggest that the disease is probably due to non-genetically determined factors.

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the concordance rate in identical twins exceeds that in nonidentical twins. A genetic effect is suggested when the impact of genetic factors on disease.

The majority of identical twins with an autoimmune disease have an unaffected twin; that is, they are discordant for the disease (Table 1). While the initially unaffected twin may develop the clinical disease some years after the index twin, the majority remain unaffected on prospective study even, as with IDDM, 40 years after the clinical diagnosis of the index twin. Nevertheless, even identical twins can differ genetically: X-chromosome inactivation in females can lead to different patterns of mosaicism; differential methylation of CpG islands can result in repression of transcription; and novel somatic rearrangements are involved in the development of T-cell receptors (TCRs) and antibodies. Thus, discordance between identical twins may be determined by nongenetic (epigenetic) factors operating on genetic expression.

Having established that environmental factors are important, the next step would be to attempt their characterization. Studies in MS have shown that nonidentical twins have a similar concordance rate to their non-twin siblings. Since nonidentical twins are expected to share a closer environment than non-twin siblings, these observations suggest that clustering of diseases in families (familial aggregation) is genetic and not environmental. Furthermore, this introduces the important distinction, confirmed by studies on adoptees and half-sibs, between environmental factors that determine the familial risk of a disease (most probably with limited influence compared with genetic factors) and environmental factors that act at the population level (and are strong determinants of disease risk). While disease-discordant identical twins should be the perfect test-bed to identify such critical environmental factors, no disease-determining environmental factor has, as yet, been found using twin studies.

The impact of genetic factors on disease
Twin studies can also be used to estimate the impact of genetic factors on the cause of a disease. A genetic effect is suggested when the concordance rate in identical twins exceeds that in nonidentical twins. This is the case for all the diseases listed in Table 1, where identical twins are more often concordant for the disease than are nonidentical twins, indicating that genetic factors are important. The concordance rates in nonidentical twins are less than 50% of those of identical twins, suggesting a polygenic model for these major immune-mediated diseases (in the case of a single dominant gene, the risk for nonidentical twins would be half that of identical twins; with increasing numbers of genes, the difference between identical and nonidentical twins would be increasingly higher). However, even infectious diseases such as polio or tuberculosis show differences in identical and nonidentical twin pair concordance rates (36% and 6% respectively for polio, and 51% and 26% respectively for tuberculosis). Taken together, these studies are consistent with genetic susceptibility influencing the appearance of clinical disease even when environmental factors play a major role in causing the disease. This conclusion is further strengthened by twin studies.

The impact of HLA genes on autoimmune disease
An association between major histocompatibility complex (MHC) HLA type and disease has been noted in many autoimmune conditions. The relative contribution of the HLA locus to disease can be estimated by comparing concordance rates in HLA-identical but dizygotic twins (or siblings) with that in monozygotic twins. Alternatively, the impact of a gene on the development of a disease (penetrance) can be estimated by comparing susceptibility genes in identical twin pairs who are discordant and concordant for the disease.

AS is strongly associated with HLA-B27. In one study, 6/8 (75%) HLA-B27+ identical twin pairs were concordant for the disease, as compared with only 4/32 (12.5%) nonidentical twins and 4/15 (27%) HLA-B27- nonidentical twin pairs. No nonidentical twin pairs have been reported to be concordant for AS but discordant for HLA-B27. These observations suggest that HLA-B27 is almost essential to the development of AS but that other genetic, as well as environmental, factors determine which HLA-B27+ individuals will manifest the disease.

Table 1. Concordance rates in identical and non-identical twin pairs in population-based studies of immune-mediated diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Identical twin pairs (%)</th>
<th>Nonidentical twin pairs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>26.7</td>
<td>3.5</td>
</tr>
<tr>
<td>RA</td>
<td>12.8</td>
<td>3.5</td>
</tr>
<tr>
<td>IDDM</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td>SLE</td>
<td>33</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: IDDM, insulin-dependent diabetes mellitus; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

The concordance rates are consistently less than 100% and are higher in identical than nonidentical pairs.
A similar suggestion can be drawn from twin studies in RA, as demonstrated by the importance of the HLA-DR shared epitope for disease concordance and the gene-dose effect in RA susceptibility. The potential of twin studies to define the role of HLA and non-HLA genes has not been exploited in other immune-mediated diseases, the main problem being the limited numbers of twin pairs available for study. Developments in population-based twin ascertainment allied to the identification of those non-HLA genes that are probably involved in disease susceptibility could lead to the wider application of twin studies in genome research.

**Limitations of twin studies in assessing genetic impact on disease**

There are other problems with assessing the genetic contribution to disease using the twin study method. First, identical twins may have a more similar environment both in utero and in childhood, which would lead to an over-estimate of heritability. Second, in contrast to nonidentical twins, identical twins are always the same sex. Many autoimmune diseases have a clear sex bias; for example, RA, MS, SLE and autoimmune thyroid disease are more common in females. Third, twin studies are hindered by potential biases in ascertainment. In the traditional ‘clinic-based’ approach, identical twin pairs discordant for a disease, or with severe disease, are more likely to be identified. In the ‘population-based’ approach, individuals are identified first as twins and are then assessed for illness. These population-based studies must ascertain large numbers of twin pairs to detect sufficient numbers of affected twins. Finally, most twin studies have been cross-sectional, but, by following twins for a longer period, it might be possible to detect a higher concordance rate.

**Immunological studies**

**Humoral immune responses**

Identical twins show a similar genetic regulation of the production of antibodies, even when they are reared apart. A recent study confirmed that such a genetic control remains largely unaltered by chronic autoimmune stimulation. Moreover, genetic factors can regulate the level and extent of antibody production. Autoantibodies are correlated with certain HLA types (e.g. HLA-DR4 and insulin autoantibodies or rheumatoid factor). A study of IgM and IgG rheumatoid factor isotypes in 70 identical and 84 nonidentical twins discordant for RA concluded that genetic factors were important in determining the levels and frequency of these isotypes; for example, IgM and IgG positivity was higher in healthy identical than healthy nonidentical twins. In agreement with such a genetic effect, combinations of IDDM-associated autoantibodies were more often found in healthy identical than healthy nonidentical twins of IDDM patients.

Other studies in immune-mediated diseases highlight differences between healthy and diseased co-twins, implying post-zygotic, epigenetic or environmental factors influencing the induction of autoantibodies. In HLA-DR4 identical twin pairs discordant for RA, the twins with RA had higher titles of antibodies towards proteins containing KGRAA, an amino acid sequence in the third hypervariable region common to the HLA-DRB1 alleles associated with the disease. In IDDM, disease-discordant identical twins are often also discordant for disease-associated autoantibodies. Moreover, autoantibodies are as often detected in healthy identical as non-identical twins of patients with an autoimmune disease, consistent with a common environmental effect; for example, IDDM-associated autoantibodies were detected in 6/18 identical twins and 6/18 nonidentical twins of IDDM patients.

**Cellular immune responses**

Cellular immune responses are under strong genetic influence. However, the peripheral TCR repertoire is shaped not only by the availability of germline receptor elements, but also by their rearrangement, thymic selection and clonal stimulation. Studies in identical twins may help define the relative importance of genetic and epigenetic factors on the shaping of the healthy (Table 2) and diseased (Table 3) immune repertoire.

Concerning the shaping of the healthy TCR repertoire, shared complementarity-determining region 3 (CDR3) motifs could be identified between healthy identical twins in the response to epitopes of myelin basic protein (MBP) and of Mycobacterium bovis 65 kDa heat shock protein. Similarly, the Vβ repertoires of identical twins were found to be more similar than those of unrelated individuals. Although shared epigenetic factors cannot be ruled out, these results may be interpreted as suggesting a predominant genetic effect. However, they contrast with a later report, again in healthy identical twins, where a common TCRVβ and/or TCRVγ gene usage for shared epitope recognition by MBP-specific T-cell lines was infrequent and there was no significant intrapair concordance, stressing the importance of epigenetic events. In accordance with this view, epitope recognition of MBP-specific T-cell lines in healthy identical
repeated stimulation either with MBP or tetanus toxoid28.

and functional characteristics of CD4

discordant for an autoimmune disease lies not in the T-cell epitope

Perhaps a similar study, performed serially over a two- or three-year

already known from previous studies of MS to be immunodominant.

epitopes of MBP recognized most frequently by the T-cell lines were

(MBP): identical twins can show differences in TCR V

toire has also come from studies on the putative antigen in MS

alterations of the TCRBV-BJ combination repertoire in the affected

cotypic DNA changes, epigenetic events or environmental factors may account for such
discordance in the TCR gene usage.

Disappointingly, twin studies have failed to identify disease-

expression was not influenced by RA (Ref. 22).

Overall, the above data suggest that the gene expression of TCRs

by peripheral lymphocytes is largely regulated by genetic factors. However, alterations in the TCR repertoire may occur both in

healthy identical twins and in the affected co-twin of pairs dis-

cordant for immune-mediated disease. Post-zygotic DNA changes, epigenetic events or environmental factors may account for such discordance in the TCR gene usage.

It is possible that the critical difference between identical twins
discordant for an autoimmune disease lies not in the T-cell epitope

expression not influenced by RA22

Perhaps a similar study, performed serially over a two- or three-year

period, will be able to clarify whether responses that remain stable

ever time differ between affected and non-affected twins.

Predicting disease

Since the healthy identical twin of patients with an autoimmune dis-

ease is at high risk of developing the same disease, it has been poss-
able to study twins before the onset of clinical disease. Evidence that

IDDM results from an environmental event operating in early child-

hood derives in part from such twin studies25. Indeed, twin studies

first revealed the chronic nature of the autoimmune process in the

prediabetic period27. These studies showed immune changes,

including disease-associated antibodies in peripheral blood, months

even years before the clinical onset of diabetes. The immune

changes were accompanied by a progressive decline in insulin secre-
tory capacity, consistent with a gradual and progressive destruction

of the insulin-secreting islet cells. A recent study suggested that the

variation in the age at clinical onset of IDDM and, by implication, the

disease incubation period following the early induction of autimmunity, is strongly genetically influenced2. The age of onset in 116

identical twin pairs discordant for IDDM was strikingly correlated

(correlation coefficient 0.94) and the correlation for age at diagnosis

was higher in identical than nonidentical twins.

Extensive twin studies of the co-twins of patients with IDDM

have revealed numerous disease-related immune changes with vari-
able predictive power. Changes that increase the risk of developing IDDM in such twins include autoantibodies to islet antigens, acti-
vation of T cells (particularly CD8+ T cells), increased expression of CD86RA (a marker of naive T cells), increased serum levels of macro-

phage-derived cytokines, impaired glucose tolerance and decreased insulin response to intravenous glucose challenge29–31. The positive

predictive value of combinations of disease-related autoantibodies is

as high as 100% (Ref. 18). In broad terms, the predictive power of immune changes in identical twins has been higher than that in

siblings, giving us an indicator of the potential of combining genetic

Table 3. Influence of epigenetic factors on the immune repertoire of individuals with immune-mediated diseases

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<tr>
<th>Condition</th>
<th>Influence on immune repertoire</th>
<th>No influence on immune repertoire</th>
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<tr>
<td>IDDM-discordant identical twins</td>
<td>Different T-cell Vβ repertoire26</td>
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<tr>
<td>RA-discordant identical twins</td>
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<td>Jβ1 expression not influenced by RA25</td>
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<td>MS-discordant identical twins</td>
<td>Different Vα usage in response to antigens28</td>
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Abbreviations: IDDM, insulin-dependent diabetes mellitus; MS, multiple sclerosis; RA, rheumatoid arthritis; for other abbreviations, see footnote to Table 2.
and immune markers in predicting IDDM in both family and popu-
lation studies. However, in twins of IDDM patients, not all immune,
and even metabolic, changes inevitably result in diabetes. Thus,
increased levels of activated T cells had a positive predictive value of
only 60%, a decrease in the first-phase insulin response to intravenous
glucose gave a positive predictive value of 58% and impaired glucose
tolerance only 35% (Ref. 33-36).

Owing to the small numbers of twins available for study, it has
been difficult to extend these observations in twins of IDDM
patients to other autoimmune diseases. In MS, cerebrospinal fluid
immunoglobulin abnormalities (oligoclonal bands), magnetic reso-
nance imaging abnormalities and visual evoked response alterations
are frequently detected in the healthy co-
twin. While the predictive value of such abnormalities is still uncer-
tain, these studies, taken together, suggest that the pathological
process associated with autoimmune conditions encompasses a
spectrum of immune changes and target organ damage that do not
inevitably lead to clinical disease. Moreover, the identification of a
long prodrome before the onset of clinical symptoms in IDDM raises
the possibility that we can predict, and possibly prevent, many of
these diseases.

Future developments

If the potential of twin studies is to be realized, a spirit of close col-
laboration must exist between clinicians and scientists, including
epidemiologists, immunologists and geneticists. To date, twin stud-
ies have provided a patchy outline of immune responses associated
with autoimmune diseases, usually in limited numbers of twin pairs
with little or no prospective analysis. Important issues that twin
studies could resolve, such as the genetic impact on disease severity
(to explain the variation between patients for arthritis, neurological
deficit or vascular damage), have not been addressed. To improve
epidemiological and immunological twin research it will be neces-
sary to increase the numbers of pairs under study. This can be attained
through the construction of population-based twin registries. Scan-
dinavian countries have a long-standing tradition in this respect and
similar registries are now being assembled in other countries42.
The creation of centralized facilities for the storage of large amounts
of frozen peripheral blood mononuclear cells and sera should be an
early goal in establishing these registries, considering also that this
would increase the chances of sampling the material from subjects
free of any therapy.

Recent technological progress highlights the potential of twin
studies: a prelude to these developments has been the successful
application of techniques such as the differential display of thou-
sands of genes to quantify gene expression; for example, in the study
of identical twins discordant for MS (Ref. 41). This analysis has led
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