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MASTERCLASS

Twin studies for the prognosis, prevention and treatment of musculoskeletal conditions

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Abstract
Background: Musculoskeletal conditions are highly prevalent in our ageing society and are therefore incurring substantial increases in population levels of years lived with disability (YLD). An evidence-based approach to the prognosis, prevention, and treatment of those disorders can allow an overall improvement in the quality of life of patients, while also softening the burden on national health care systems.
Methods: In this Masterclass article, we provide an overview of the most relevant twin study designs, their advantages, limitations and major contributions to the investigation of traits related to the domain of musculoskeletal physical therapy.
Conclusions: Twin studies can be an important scientific tool to address issues related to musculoskeletal conditions. They allow researchers to understand how genes and environment combine to influence human health and disease. Twin registries and international collaboration through existing networks can provide resources for achieving large sample sizes and access to expertise in study design and analysis of twin data.

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Background

In 2003, it was predicted that the global burden of major musculoskeletal conditions on individuals and society would raise dramatically in the following years, primarily due to the ageing of the population.[1] These expectations have been recently confirmed. According to the Global Burden of Disease Study 2015, musculoskeletal disorders were the largest contributors to years lived with disability (YLD).[2] From 2005 to 2015, there have been increases of 17.3%, 23.8% and 32.9% in the prevalence of low back pain, rheumatoid arthritis and osteoarthritis, respectively, in the world population.[2]

In the United States alone, the four aforementioned conditions represented an economic impact of $796.3 billion dollars in direct costs between 2009 and 2011.[3] The consequences of the substantial increase in the prevalence of musculoskeletal diseases on the national public health care systems are still unclear, but an evidence-based approach to prognosis, risk prediction, prevention and treatment strategies is highly needed. Traditional epidemiological studies testing associations between these outcomes and certain exposures can be informative but do not allow for disentangling genetic and environmental factors contributing to disease. Genetic epidemiology provides means for this through a specific type of family study design – the twin study.

Twin studies have been playing an important role in improving the understanding of human traits and conditions since the late 19th century,[4] although their first applications in musculoskeletal research, to our knowledge, date back only to the late 1980s and early 90s.[5–8] At that time, twin registries collecting valuable longitudinal data had already been established in Denmark,[9] Sweden,[10] Finland,[11] USA[12] and Australia.[13] Newly developed statistical techniques in multivariate modelling[14] and analysis of gene by environment interactions[15] provided extended uses of the classical and co-twin control method. Twin study designs had been classified into those investigating the genetic factors contributing to disease and those 'excluding', or adjusting for, genetic factors, thus useful to assess etiologic importance of environmental factors,[16] a distinction that is still reasonably clear in current approaches.

Twin studies have become even more relevant with the advent of new technologies and analytical methods.[17] This article aims to discuss the existing twin designs, their potential and contributions to the musculoskeletal scientific domain, while also highlighting the advantages and potential caveats of the twin methodology. We also suggest how global research networks, which are being formed with the help of twin registries,[18] can be used for collaborative work. We claim that musculoskeletal research has benefited greatly from twin studies, although there is still considerable unfulfilled potential in utilising this methodology especially for studying risk factors and treatments for disease. We also point to plausible directions for future applications of the twin methodology in musculoskeletal studies.

The advantages of twin designs and applications in musculoskeletal research

Twin studies have been historically associated with the study of human genetics since Francis Galton established the 'nature vs nurture' debate in the late 19th century.[4] Although Francis Galton is commonly regarded as the 'father' of twin research, it is unclear whether he was aware of the distinction between monozygotic (MZ) twins, also known as identical, and dizygotic (DZ) twins, also known as fraternal twins. He did notice 'similar' and 'dissimilar' twins, bud did not seem to account for genetic differences between these two types of twins.

At some point in the early 20th century, researchers seem to have agreed on the traditional theory of twinning – that MZ twins originate from a single fertilised egg while DZ twins originate from two different fertilised eggs.[19]

The comparisons within MZ twin pairs with those within DZ pairs for a specific human trait is the foundation of the classical twin design. This model assumes that MZ pairs share, on average, 100% of their genetic variation, compared to DZ pairs, which would share 50% of their genetic variation as in any normal pair of siblings, based on the principles of Mendelian inheritance.[20] It also assumes that the common environment in which both MZ and DZ twin pairs are reared is the same. This is also known as the equal environment assumption (EEA), which is overlooked by researchers in some occasions.[21]

Using this study design, most researchers are interested in the narrow-sense heritability score (h^2), an outcome that is an estimate of the proportion of variance in a trait that can be explained by additive genetic variation.[22] The heritability estimate can range from 0 to 1. If MZ twin pairs are significantly more similar than DZ pairs, then it is assumed that there is genetic influence explaining the variation of that trait in a population. It is important that the correlation and co-variance estimates themselves are also considered in the analysis.

The classic twin model also sheds light on all non-genetic influences in the variation of a trait, including environmental and stochastic factors along with potential measurement errors. The proportion related to those just cited can be calculated by $1 - h^2$. Perhaps one of the most notable early applications of the classical twin design in musculoskeletal research was a pioneering study that demonstrated the strength of genetic contributions to the bone mass of the lumbar spine in adults, finding a statistically significant heritability score of 0.92.[5] Intra-class correlations in this trait were 0.92 for MZs against 0.36 for DZ twins. Correlations of that magnitude within genetically identical twin pairs for such an important trait have important implications for health care. Other highly impactful classical twin studies from late last century include a classical twin study that found a heritability of 0.54 for combined types of osteoarthritis in women aged 48–70 recruited from a twin registry in the UK, showing for the first time the genetic effect on the condition, but also stressing the importance of environmental influences.[23]

More recently, a systematic review of twin studies assessing low back pain as an outcome reported the heritability as ranging from 21% to −67%, while in a younger age group (11 years old), the heritability was as low as 0%.[24] The authors concluded that the variability amongst studies can be related to differences in how heritability is calculated, low back pain assessment and age, although a clear pattern on age-dependant effects did not emerge. The systematic review also included twin studies that investigated a

number of associations between exposures, conditions and co-morbidities, in a study design usually referred as the co-twin control design.

The co-twin control design's mains strength is the possibility of matching cases and controls within twin pairs for genes, early environment, age and sex, depending on the zygosities included.[25] Although this study design is mostly aimed at the participation of MZ twin pairs to achieve a very high level of matching, the inclusion of same-sex DZs can be also informative. Relevant analytical approaches include studying twin pairs discordant for exposures or outcomes.

Highly impactful twin studies utilising this particular design have been conducted to examine musculoskeletal conditions. One example is a twin study that aimed to investigate the association between obesity and osteoarthritis of the hand and the knee in women, who have an overall higher risk for this condition than men.[26] The study found that obesity is indeed a risk factor for OA, with increased risks of 9–13% of developing the disease depending on the magnitude of weight gain. Interestingly, confidence intervals were considerably narrower than in studies conducted with general population,[27] indicating the statistical advantages arising from the smaller variability typically found when using a twin sample in a case–control design.

Both the classical and the co-twin control designs can be used in cross-sectional and longitudinal studies. The latter is more promising regarding inferences on causation and involve less recall bias. A recent longitudinal co-twin control study found no significant association between obesity (including BMI, % fat mass, waist circumference and waist-to-hip ratio as exposure variables) and low back pain.[28] The results of the study point to the relationship between obesity and low back pain being potentially confounded by familial factors, since the association is not confirmed when the model adjusts for genetics as a confounder. A previous cross-sectional co-twin control study on low back pain identified some possible risk factors, mostly related to physical workload, such as heavy lifting, manual tasks and gardening.[29]

The application of the classical twin design conducted longitudinally makes causal inferences possible. A study that had initially found high rates of MZ twin pairs discordant for type II diabetes at an early adult age proceeded to follow those twins and reassess them to find they eventually became predominantly concordant later in life.[10] Although unrelated to musculoskeletal research, this study helped highlight the importance of understanding heritability as a snapshot of potential genetic causes of variation in a trait or disease in specific populations and points in time, rather than evidence of direct causation.

A recent study demonstrated how age-specific heritability estimates can differ between cross-sectional and longitudinal analyses. While cross-sectional heritability estimates of hip and lumber spine bone mass density decreased but remained consistently high from early post-menopausal to late ages in women, a longitudinal analysis indicated that virtually none of the variation in those traits was explained by genetic variation in some age groups.[30]

Longitudinal designs involving twins as participants can be applied also to analyses of survival, mortality and co-morbidity. By using intrapair Cox regression models, a twin study found that after one year of exposure to hip fracture, men had a seven-fold increase in mortality, compared to a four-fold increase amongst women.[31] In another example, researchers found spinal pain to be associated with a 13% increased rate in all-cause mortality.[32]

Another application of the within-pair analysis is its use in clinical trials, a design in which the involvement of twins is especially advantageous.[33] In some cases, the use of the within-pair analysis in studies with MZ twins can lead to up to seven times more statistical power to detect effects of interventions.[34] Randomised control trials (RCTs) making use of the co-twin control design have shown to be feasible and potentially rewarding, as in a study which found Calcium supplementation to be effective in increasing bone mineral density over the first 12–18 months of treatment, although gains were not maintained after 24 months[35] and in the ongoing intervention on sleep quality to improve outcomes in low back pain.[36] RCTs with twins are promising, but remain underutilised to this date in research involving twins as participants.

Other promising twin study designs

The study of sex differences in traits and diseases can also be accomplished through twin designs, with the opposite-sex twin design probably the most efficient example. An opposite-sex twin study found differences between men and women in the nature of the association between peptide hormones responsible for adiposity and body composition outcomes.[37] While in women plasma ghrelin hormone levels were inversely correlated with BMI and total fat mass, in men there was an observed inverse correlation between the hormone and abdominal fat mass. Sex differences were also investigated using multivariate twin model in a cohort study of Danish twins, which found women at higher risk of primary total knee arthroplasty after 50 years old than men.[38]

Structured equation modelling (SEM) has been developed for applications in an extended version of the classical twin design. The ACE method partitions the variation of a trait into A (additive genetics), C (common environment), and E (unique environment) estimates, and fits the best possible model within those three components.[14] A study using this design found little genetic influence in the variation in physical activity levels, which was found to be mostly explained by common and unique environmental factors[39] and contradicted previous findings of strong genetic influence.[40] Even more interestingly, both studies also differed in the cut-points of determining whether twins were or not exercisers. Heritability estimates seemed to have been substantially attenuated when a weekly 150 min cut-point was used compared to a 60 min weekly cut-point. This might have shown consistency with a gene by environment interaction, since genetic influence looked different for distinct levels of exposure.

The analytical techniques to test for gene by environment interactions were developed in 1970,[15] although to our knowledge, there has not been any direct application of it to twin studies looking at musculoskeletal conditions. More sophisticated analysis related to quantitative genetics allowed by the conclusion of the Human Genome Project in the 1990s can also help find candidate genes for traits and diseases. Recent accounts from The Twin Spine Study[41] show evidence of association between 17 different genes

and lumbar disc degeneration, although some of the findings still lack proper replication. It also pointed to a strong genetic influence in the variation in this condition.

Potential caveats

Twin designs rely heavily on assumptions, as with any other statistical model. As previously explained, the first assumption is that MZ twin pairs share, on average, 100% of their genetic variation, compared to only 50% for DZ twin pairs. Cases of DZ twins sharing more or less than this have been found and the traditional twining model of how MZ and DZ twins are originated has been disrupted (but only rarely) by observation of atypical twinning.[42] However, in large samples the 50/100 rule has remained consistent and still applies.[17]

The second assumption concerns the extent to which twins share the early environment, which is set to be 100%, equally for MZ and DZ twin pairs. The early environment can include several unmeasured factors such as those related to sharing the same womb and in most cases the same early-life environment, defined by parenting style, schools attended by the twins and nutritional aspects, for example. This assumption, known as EEA, has received scrutiny over the years, such as in claims that simply estimating heritability brings unsubstantial evidence for problems relating to human genetics.[43] Methods to account for disruptions to the EEA, such as differences in cohabitation within twin pairs[44] have also been proposed.

The misinterpretation of heritability estimates can also be an issue, especially when these lead to biased policy-making and public discussion.[45] It is important to account for the difference between causes of variation in a trait and causes of a trait, as it has been shown that they can mean very different things in some scenarios. For example, when investigating hypothetical scenarios, populations with differences in average energy intake and levels of exercise would show different heritability estimates for obesity, although not much could be then said about genetics actually having or not a causal effect on obesity.[46]

Whether twins are representative of the population they are drawn from deserves additional attention. For most disease and lifestyle-related traits they are quite similar to the general population, and potential differences could be attributed to preterm birth and low birth weight which are more prevalent in twins compared to singletons.[47]

Twin registries and international collaboration

Although sample size was once considered a barrier for achieving robust scientific findings through twin studies, the current era of international collaboration and joint studies with multiple twin registries offer potential solutions to this problem. Twin registries have been playing a central role in addressing problems with sample size in twin studies, especially since statistical calculations showed how large twin studies needed to be to achieve robust findings.[48] These registries have shown their relevance in investigating rare diseases with the use of twin designs, or even in finding large enough samples of twins that are discordant for outcomes or exposures as in co-twin control studies, especially when the traits being investigated are highly heritable. They have also been supporting multidisciplinary research focused on addressing problems in a variety of health-related domains, such as in cardiovascular disease.[49]

Table 1 Characteristics of registered twin pairs with baseline data in the Brazilian Twin Registry.

Characteristics	Number (percentage or SD)
Total number of complete twin pairs with baseline data	108
Monozygotic twin pairs	88 (81.5%)
Male–male	17 (19.3%)
Female–female	71 (80.7%)
Dizygotic twin pairs	20 (18.5%)
Male–male	2 (10.0%)
Female–female	7 (35.0%)
Opposite-sex	11 (55.0%)
Age (mean)	30.0 (12 SD)

In order to register a larger number of twins, twin registries and cohorts have relied upon a variety of recruitment strategies. A special issue of the journal Twin Research and Human Genetics on twin registries highlighted that strategies could be population and/or volunteer-based,[50] which have implications for obtaining samples that are similar enough to the general population for participating in a scientific study. One study concluded that response rates from twins as volunteers in Australia were similar to non-twin volunteers.[21]

Regardless of the recruitment strategies used in twin registries, in some cases a national sample of twins might not be sufficient to achieve the desired sample size. Twin registries have organised to form the International Network of Twin Registries,[18] established in 2011 as a working group of the International Society for Twin Studies. This network aims to facilitate collaboration and promote twin research globally, and is currently building a catalogue of available twin data and biospecimens that are available for ethically approved studies, while also profiling researchers interested in forging new collaborations.[51]

International collaboration in twin research has already generated studies that included more than 200,000 twin pairs, such as the CODATwins project, which mostly investigates trends in anthropometric variables.[52] Another trend in these collaborative efforts in twin studies in musculoskeletal research are studies including data linkage between twin registries and other registries, such as those of arthroplasty and knee replacement.[53]

Even after more than 50 years since the establishment of the first twin registries, such organisations continue to be established and funded, including countries in which twin research has not historically taken a major role. For example, the Brazilian Twin Registry was established in 2013 and has 108 complete pairs with baseline data (see Table 1). It is focused on studies within the musculoskeletal domain and is currently recruiting for a study on the environmental factors playing a role in low back pain.[54] The registry is also open for new collaborations and takes enquiries from researchers wanting to undertake twin studies with its support.

Conclusions and future research

About 30 years have passed since the first twin studies on musculoskeletal conditions were published and, to this date, the involvement of twins as participants has facilitated an evidence-based approach towards prognosis, prevention and treatment. Although each twin design has its advantages and disadvantages, the analysis of within-pair differences can be an especially relevant tool for researchers investigating associations between environmental factors and related outcomes with the potential of more reliable scientific outcomes due to epidemiological matching. For treatment, RCTs involving twins being allocated to different treatment groups can also generate results that can be more directly translated into clinical practice. Extended family designs including relatives of twins in studies are also very promising, although still largely unexplored in the musculoskeletal domain.

With growing international collaboration between researchers and twin registries, it is now becoming more convenient not only to access existing data and biospecimens of twins for novel analyses but also to form consortia and recruit twins for new studies in multi-centre approaches. Twin registries have become more relevant than ever to elucidate risk, prognosis, and treatment factors for musculoskeletal conditions, bringing twins and researchers together to better understand what it is to be human.

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Conflicts of interest

None declared.

References

1. Anthony W, Bruce P. Burden of major musculoskeletal conditions. *Bull World Health Organ*. 2003;9:646–656.
2. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;(10053):1545.
3. Yelin E, Weinstein S, King T. The burden of musculoskeletal diseases in the United States. *Semin Arthritis Rheum*. 2016;(3):259.
4. Galton F. The history of twins, as a criterion of the relative powers of nature and nurture. *J Royal Anthropol Inst*. 1876:391.
5. Pocock NA, Eisman JA, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults: a twin study. *Aust N Z J Med*. 1987;17(suppl 1).
6. Bengtsson B, Thorson J. Back pain: a study of twins. *Acta Genet Med Gemellol (Roma)*. 1991;40(1):83–90.
7. Silman AJ, MacGregor AJ, Thomson W, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol*. 1993;32(10):903–907.
8. Hopper JL, Seeman E. The bone density of female twins discordant for tobacco use. *N Engl J Med*. 1994;(6):387.
9. Hauge M, Harvald B, Fischer M, et al. The Danish twin register. *Acta Genet Med Gemellol (Roma)*. 1968;17(2):315–332.
10. Cederlöf R, Floderus B, Friberg L. The Swedish twin registry – past and future use. *Acta Genet Med Gemellol (Roma)*. 1970;19(1):351–354.
11. Kaprio J, Sarna S, Koskenvuo M, Rantasalo I. The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Prog Clin Biol Res*. 1978;24(PtB):179–184.
12. Jablon S, Neel JV, Gershowitz H, Atkinson GF. The NAS-NRC twin panel: methods of construction of the panel, zygosity diagnosis, and proposed use. *Am J Hum Genet*. 1967;19(2):133–161.
13. Clifford CA, Hopper JL. The Australian NHMRC Twin Registry. A resource for the Australian scientific community. *Med J Aust*. 1986;145(2):63–65.
14. Hopper JL, Mathews JD. Extensions to multivariate normal models for pedigree analysis. *Ann Hum Genet*. 1982;46(Pt 4):373–383.
15. Jinks JL, Fulker DW. Comparison of the biometrical genetical, MAVA, and classical approaches to the analysis of human behavior. *Psychol Bull*. 1970;73(5):311–349.
16. Hrubec Z, Robinette CD. The study of human twins in medical research. *N Engl J Med*. 1984;310(7):435–441.
17. van Dongen J, Slagboom PE, Draisma HHM, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nat Rev Genet*. 2012;(9):640.
18. Buchwald D, Kaprio J, Hopper JL, et al. International Network of Twin Registries (INTR): building a platform for international collaboration. *Twin Res Hum Genet*. 2014;17(6):574–577.
19. Hall JG. Twinning. *Lancet*. 2003;(9385):735.
20. Mendel G. In: Bennett JH, ed. *Experiments in plant hybridisation: Mendel's original paper in English translation, with commentary and assessment by the late Sir Ronald A. Fisher, together with a reprint of W. Bateson's biographical notice of Mendel/Gregor Mendel*. Edinburgh: Oliver & Boyd; 1965.
21. Hopper JL. The epidemiology of genetic epidemiology. *Acta Genet Med Gemellol (Roma)*. 1992;41(4):261–273.
22. Burton PR, Tobin MD, Hopper JL. Series: key concepts in genetic epidemiology. *Lancet*. 2005;366:941–951.
23. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D, Hopper JL. Genetic influences on osteoarthritis in women: a twin study. *BMJ*. 1996;(7036):940.
24. Ferreira PH, Beckenkamp P, Maher CG, Hopper JL, Ferreira ML. Nature or nurture in low back pain? Results of a systematic review of studies based on twin samples. *Eur J Pain*. 2013;17(7):957–971.
25. Duffy D. The co-twin control study. In: *Advances in Twin and Sib-Pair Analysis.*; 2000.
26. Cicuttini FM, Baker JR, Spector TD. The association of obesity with osteoarthritis of the hand and knee in women: a twin study. *J Rheumatol*. 1996;23(7):1221–1226.
27. Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis The Framingham Study. *Ann Intern Med*. 1988;109(1):18–24.
28. Dario AB, Loureiro Ferreira M, Refshauge K, Luque-Suarez A, Ordoñana JR, Ferreira PH. Obesity does not increase the risk of chronic low back pain when genetics are considered. A prospective study of Spanish adult twins. *Spine J*. 2017;17(2):282–290.
29. Oliveira VC, Ferreira ML, Refshauge KM, et al. Risk factors for low back pain: insights from a novel case–control twin study. *Spine J*. 2015;15(1):50–57.
30. Moayyeri A, Hammond CJ, Hart DJ, Spector TD. Effects of age on genetic influence on bone loss over 17 years in women:

the Healthy Ageing Twin Study (HATS). *J Bone Miner Res*. 2012;27(10):2170–2178.
31. Michaelsson K, Nordstrom P, Nordstrom A, et al. Impact of hip fracture on mortality: a cohort study in hip fracture discordant identical twins. *J Bone Miner Res*. 2014;29(2):424–431.
32. Fernandez M, Boyle E, Hartvigsen J, et al. Is this back pain killing me? All-cause and cardiovascular-specific mortality in older Danish twins with spinal pain. *Eur J Pain*. 2017;21(5):938–948.
33. Plomin R, Haworth CMA. Genetics and intervention research. *Perspect Psychol Sci*. 2010;5:557.
34. Carr AB, Martin NG, Whitfield JB. Usefulness of the co-twin control design in investigations as exemplified in a study of effects of ascorbic acid on laboratory test results. *Clin Chem*. 1981;27(8):1469–1470.
35. Cameron MA, Paton LM, Nowson CA, Margerison C, Frame M, Wark JD. The effect of calcium supplementation on bone density in premenarcheal females: a co-twin approach. *J Clin Endocrinol Metab*. 2004;89(10):4916–4922.
36. Pinheiro MB, Ho KK, Ferreira ML, et al. Efficacy of a sleep quality intervention in people with low back pain: protocol for a feasibility randomized co-twin controlled trial. *Twin Res Hum Genet*. 2016;19(5):492–501.
37. Makovey J, Naganathan V, Seibel M, Sambrook P. Gender differences in plasma ghrelin and its relations to body composition and bone – an opposite-sex twin study. *Clin Endocrinol (Oxf)*. 2007;66(4):530–537.
38. Skousgaard SG, Skytthe A, Moller S, Overgaard S, Brandt LP. Sex differences in risk and heritability estimates on primary knee osteoarthritis leading to total knee arthroplasty: a nationwide population based follow up study in Danish twins. *Arthritis Res Ther*. 2016;18:46.
39. Duncan GE, Goldberg J, Noonan C, Moudon AV, Hurvitz P, Buchwald D. Unique environmental effects on physical activity participation: a twin study. *PLoS ONE*. 2008;3(4):e2019.
40. Stubbe JH, Boomsma DI, Vink JM, et al. Genetic influences on exercise participation in 37,051 twin pairs from seven countries. *PLoS ONE*. 2006;1:e22.
41. Battié MC, Videman T, Kaprio J, et al. The Twin Spine Study: contributions to a changing view of disc degeneration. *Spine J*. 2009;9(1):47–59.
42. McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP. A review of the mechanisms and evidence for typical and atypical twinning. *Am J Obstet Gynecol*. 2016;214(2):172.
43. Feldman MW, Lewontin RC. The heritability hang-up. *Science*. 1975;(4220):1163.
44. Hopper JL, Culross PR. Covariation between family members as a function of cohabitation history. *Behav Genet*. 1983;13(5):459–471.
45. McLean D, Pearce N, Walls CB, Wigley RD. The ''Twin Study'' and the misunderstanding of epidemiology that clouds occupational associations and low back disorder. *N Z Med J*. 2011;124(1339):109–111.
46. Pearce N. Epidemiology in a changing world: variation, causation and ubiquitous risk factors. *Int J Epidemiol*. 2011;40(2):503–512.
47. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res Hum Genet*. 2001;(6):464.
48. Martin NG, Eaves LJ, Kearsey MJ, Davies P. The power of the classical twin study. *Heredity (Edinb)*. 1978;40(1):97.
49. Ferreira LC, Craig JM, Hopper JL, Carrick SE. The Charles Perkins centre's twins research node. *Twin Res Hum Genet*. 2016;19(4):393–396.
50. Hur YM, Craig JM. Twin registries worldwide: an important resource for scientific research. *Twin Res Hum Genet*. 2013;16(1):1–12.
51. Worldwide Twins. Retrieved from www.worldwidetwins.net on 14/10/2017. 2017.
52. Silventoinen K, Jelenkovic A, Sund R, et al. The CODATwins project: the cohort description of collaborative project of development of anthropometrical measures in twins to study macro-environmental variation in genetic and environmental effects on anthropometric traits. *Twin Res Hum Genet*. 2015;18(4):348–360.
53. Magnusson K, Scurrah K, Ystrom E, et al. Genetic factors contribute more to hip than knee surgery due to osteoarthritis – a population-based twin registry study of joint arthroplasty. *Osteoarthr Cartil*. 2017;25(6):878–884.
54. Ferreira PH, Oliveira VC, Junqueira DR, et al. The Brazilian twin registry. *Twin Res Hum Genet*. 2016;19(6):687–691.