

# BJO

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## Editorials

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### Bias: adding to the uncertainty

Through their exceptionally thorough follow up, Pennefather *et al* (this issue, p 643) have presented us with a fine example of the impact that bias, or systematic error, can have on the results of an epidemiological study. They found a higher rate of ocular abnormalities in children who were hard to locate or whose parents were reluctant for them to attend for follow up, suggesting that a less comprehensive survey would have underestimated the extent of disease.

Epidemiological studies are subject to two types of error, systematic and random, and of the two, the systematic errors are by far the more problematic. Statistical theory offers us an abundance of methods for quantifying and allowing for the impact of random error by using standard errors, confidence intervals, or *p* values. From this theory we know that as the sample increases so the size of the random or sampling error will decrease, often in proportion to the square root of the sample size. Bias, however, is much more difficult to handle because it is generally unmeasured and, being systematic, it does not decrease as the sample size increases. It is important to remember that a confidence interval only captures the uncertainty due to sampling errors and consequently can only be thought to represent a likely range of values for the feature of interest if we believe that there is no bias in the study.

Sackett's influential article attempted to catalogue the sources of different forms of bias that can arise.<sup>1</sup> Despite listing over 50 sources of bias in medical research these are all essentially variations on three key themes—measurement error, selection bias, and confounding. Measurement error is the most obvious type of bias and includes not only straightforward examples such as a faulty tonometer that underestimates intraocular pressure, but also problems because of forgotten exposures or misdiagnosis. Selection bias arises through the inclusion or exclusion of subjects leading to an unrepresentative sample. Pennefather *et al*'s paper illustrates this. It is a common experience that studies which are set out to recruit a representative sample fail because of selective non-response. The third form of bias, confounding, is important in comparative studies. If a study is correctly randomised then the comparison groups will tend to be similar in every respect except the allocated treatment or exposure. Observational studies are not able to randomise and so it is not possible to ensure that the comparison groups do not differ in other important respects. Thus, a comparison between a new surgical technique and previous experience of a standard procedure is open to bias

if there have been other changes in medical practice or patient selection that have coincided with the introduction of the new technique. It is possible to make a statistical adjustment to the comparison for known confounders provided that these can be measured. One of the key advantages of randomisation is that it will automatically tend to balance out potential confounders even when they are neither suspected nor measured.

Sometimes it is claimed that non-differential bias—that is, bias that affects everyone equally, will only diminish the size of associations or differences. It follows that if a study finds an effect, the removal of any non-differential bias would only increase the effect. Theoretical studies and simulations have supported this idea but in practice the situation is usually more complex. Even a small amount of differential bias can exaggerate the size of an effect and where there are more than two factors at play, bias in one can affect the apparent relation between two others. It is therefore very difficult to be sure that a result in an observational study is not due to some bias.

Even with the security of a randomised trial methodology in place an overall selection bias may exist because of excessively stringent inclusion/exclusion criteria. Thus, a trial result may be perfectly valid within itself but if the design is not pragmatic the result may lack generalisability, as a significant proportion of “real world patients” may fall outside the trial entry criteria. A quest for purity, with overemphasis of trial population homogeneity, may ultimately be counterproductive.

Given the potential for misleading results due to bias in epidemiological studies it is almost surprising that observational studies make any contribution to medical research. Indeed, it is true that no epidemiological study should be treated as convincing evidence in isolation. It is only when a number of epidemiological studies using different methodologies in different populations agree on some finding that one should be persuaded of its truth. Despite this reservation some epidemiological studies are better than others and one of the main features that must be assessed in a critical appraisal of the evidence is the way that the study attempts to minimise bias or quantify its potential impact.

Pennefather *et al* conclude their article by reiterating that the difference between the prevalence of ocular abnormalities in the cooperative subjects was not significantly different from that in the whole cohort. This appears to contradict the (valid) tests presented in their Table 1 (p 644), which show that significant differences do exist between

the three groups. The main point, however, is that when dealing with bias it is more relevant to emphasise the importance of the overall impact resulting from the bias, rather than focusing on statistically significant subgroup differences. Thus, the primary question has been appropriately addressed—that is, whether the difference between the easily measured 11.3% ocular abnormalities and the true value for this cohort of 13.4% is of practical importance.

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## Science! Why should the clinician care?

How does molecular/genetic ophthalmic research benefit the clinician? If this question had been asked 10 years ago the answer would have been quite different from that of today. At that time very few ocular genes were known. The main aim of laboratory research was to establish linkage for an inherited disorder to a specific chromosomal region for a family. This allowed the early identification of members of a family who were at risk of being affected. Even this type of work was limited to conditions such as gyrate atrophy, cataracts, and retinitis pigmentosa, because linkage work was still very much in its infancy.

In 1999 there are over 60 ocular genes identified.<sup>1</sup> A clinician can request not just linkage information but specific mutation screening for many inherited ocular diseases, ranging from corneal dystrophies to Norrie's disease. Because of limited funding, much of this work is performed at the research level only and not widely available as a laboratory service. The clinician can, however, use these data to counsel patients far more accurately and request prenatal testing.

Over the next 10 years it would be reasonable to anticipate the completion of the human genome mapping project and the identification of many more genes responsible for ocular disease. At this time the clinician might reasonably ask not only for specific mutation information but also to be advised on new therapies that might be available. In some cases this may involve gene therapy, in others it might be replacement of a protein deficient in individuals deemed to be at risk of developing the disease. Alternatively, it may be that for diverse diseases such as glaucoma, one particular subgroup—for example, GLC1A, may be shown to respond better to medical treatments than surgical. With this knowledge the clinician can instigate the best and most appropriate therapy.

Having established that a rudimentary knowledge of molecular and genetic ophthalmology is important to the clinician, it is refreshing to see research, that a couple of years ago would have been published only in the scientific journals, now appearing in the ophthalmic literature. In this issue of the *BJO*, Nishina and colleagues (p 723) discuss the pattern of expression of a gene central to the development of the eye. This work is important because it is the first time that human embryos have been studied as late as 22 weeks' gestation. Previous work has focused mainly on animal or very early human embryos.<sup>2</sup> The gene under study in this paper (*PAX6*) is fascinating because it has changed very little throughout evolution. In fact, the mouse and human gene products differ by only one amino acid.<sup>3</sup> *PAX6* is not only expressed in the eye but also in the developing pancreas and

CNS.<sup>4</sup> The regulation of *PAX6* expression is largely unknown but recent studies in mice have shown that the gene contains two distinct promoters, P0 and P1, that control the level of *PAX6* in specific tissues. For example, P0 activation produces transcripts predominately in the lens, cornea, and conjunctival epithelium, whereas P1 initiated transcripts are expressed in lens, optic vesicle, and CNS. Other regulatory elements exist within the *PAX6* gene that require stimulation for expression to occur in the pancreas or in particular subsets of retinal cells.<sup>5</sup>

The *PAX6* gene is thought to be at the top in the hierarchy of genes that determine when and how different parts of the eye differentiate. The exact pathways have yet to be elucidated but Nishina *et al* suggest some of the genes that are likely to be involved in this cascade. It is interesting that the *PAX6* gene is widely expressed in ectodermal but not mesenchymal structures because mutations in *PAX6* give rise to abnormalities in tissues derived from mesenchyme—for example, aniridia and Peters' anomaly. Nishina and colleagues propose explanations for these and other *PAX6* disorders, suggesting that the *PAX6* protein targets other genes that may be expressed in, or control the differentiation of, these tissues, such as neural cell adhesion molecule (N-CAM), crystallins, and other retinal homeobox genes. If *PAX6* is at the top of the pyramid one might expect that gene mutations could affect virtually any part of the eye via its interactions. Several *PAX6* screening studies are under way to identify mutations in a variety of different developmental conditions (GC Black, St Mary's Hospital, Manchester; IM Hanson, MRC Human Genetics Unit, Edinburgh; AJ Churchill, St James's Hospital, Leeds, unpublished data). To date, *PAX6* mutations have been found to cause aniridia, dominant keratitis, Peters' anomaly, foveal hypoplasia, and congenital cataracts.<sup>6</sup> Only a minority of cases of Peters' anomaly are, however, due to *PAX6* mutations and it will be interesting to see if screening some of the proposed target genes reveals new mutations in this and other congenital disorders.<sup>7</sup>

If *PAX6* controls and orchestrates ocular development why does expression continue in the adult corneal and conjunctival epithelium? Nishina *et al* suggest that normal *PAX6* protein may be necessary for the maintenance of a healthy cornea in that haploinsufficiency can result in keratitis. A similar picture is not infrequently seen in aniridias where minor epithelial trauma may be followed by a poor healing response with apparent stem cell failure. If a critical concentration of *PAX6* protein is required for corneal health then it may not be beyond the realms of possibility to synthesise the

protein and administer it in topical form to those patients with apparent stem cell failure.

There is a fundamental need for close collaboration between clinicians and scientists to maximise research potential. The paper by Nishina *et al* is an example of a successful multidisciplinary team effort. Clinicians must stay abreast of scientific advances to improve such links with laboratory based colleagues and thereby fully appreciate what molecular/genetic research has to offer.

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