Int. J. Dev. Biol. 43: 453-456 (1999)

Influence of fetal environment on kidney development

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ABSTRACT Several lines of evidence, mostly derived from animal studies, indicate that changes in fetal environment may affect renal development. Besides maternal hyperglycemia or drug exposure, that were recently found to alter nephrogenesis, changes in vitamin A supply to the fetus may prove to be responsible for most of the variations in nephron number found in the population. A low vitamin A status in the fetus may be a major cause of inborn nephron deficit, either as a feature of intrauterine growth retardation or independently of growth retardation. The possibility that vitamin A status may also influence renal vascular development is raised. We suggest that low vitamin A supply to the fetus plays a role in the intrauterine programming of chronic renal disease and hypertension.

KEY WORDS: *fetal environment, vitamin A status, maternal hyperglycemia, fetal exposure to drugs, renal development, intrauterine programming of adult diseases*

Introduction

Kidney organogenesis is the result of interactions between the undifferentiated metanephric blastema and the ureteric bud, a branching epithelial structure emerging from the Wolffian duct. As the tips of the ureteric bud branches interact with the metanephric blastema, they induce local mesenchymal cells to condense into clusters, and to undergo a mesenchymal-to-epithelial conversion process leading to nephron formation (Saxén, 1987). At the end of nephrogenesis, a permanent number of nephrons, specific for a given species, has formed. Nephron deficits can result from the failure of the ureteric bud to branch appropriately or from the failure of the mesenchyme to differentiate in response to inductive signals. It is now recognized that the number of nephrons in the human kidney varies more than was formerly believed, indicating substantial, though silent, nephron deficits (Brenner and Chertow, 1993; Merlet-Bénichou *et al.*, 1999a). Although *Pax-2* mutations were recently found to account for some cases of isolated oligomeganephronic renal hypoplasia (Tellier *et al.*, 1998), evidence is mounting that non-syndromic nephron deficits result from environmental changes *in utero*. A permanent nephron deficit has been found to be associated with intrauterine growth retardation in humans and animals (Merlet-Bénichou *et al.*, 1999a). This finding has stimulated speculation about the role of renal development in the intrauterine programming of chronic renal disease and hypertension that occur later in life (Brenner and Chertow, 1993; Merlet-Bénichou *et al.*, 1999a). The recent recognition that a micronutrient, vitamin A, and its main derivative, retinoic acid, closely modulate nephron number and may be involved in the development of renal vasculature, added a new dimension to the debate (Merlet-Bénichou *et al.*, 1999b). The present review discusses the role of retinoids in renal organogenesis and the pathophysiological implication of mild vitamin A deficiency. The influence on the fetal kidney of other abnormal conditions *in utero*, like hyperglycemia associated with diabetes mellitus or drug administration, is also reviewed.

Control of nephrogenesis by vitamin A

As demonstrated half a century ago in rats exposed *in utero* to severe vitamin A deficiency (Wilson and Warkany, 1948), and more recently in mice bearing a double mutation for specific combinations of retinoid nuclear receptors (Mendelsohn *et al.*, 1994), retinoids are involved in renal development. We used rat metanephric organ culture to show that retinoids stimulate nephrogenesis in a dose-dependent manner (Figs. 1A,B and 2) (Vilar *et al.*, 1996). Using a model of mild vitamin A deficiency in the pregnant rat, we also found that a 50% reduction of the circulating vitamin A in the fetus, although it did not prevent an overall normal development to occur, resulted in a 25% reduction in the number of nephrons (Lelièvre-Pégorier *et al.*, 1998b). The study allowed a positive linear correlation to be evidenced between the number of nephrons and the circulating vitamin A level in term fetuses (Fig. 3).

Remarkably, this correlation was found in vitamin A deficient fetuses as well as in the controls. Moreover, a single injection of retinoic acid given to control pregnant rats at mid-gestation was

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0214-6282/99/$15.00

Printed in Spain
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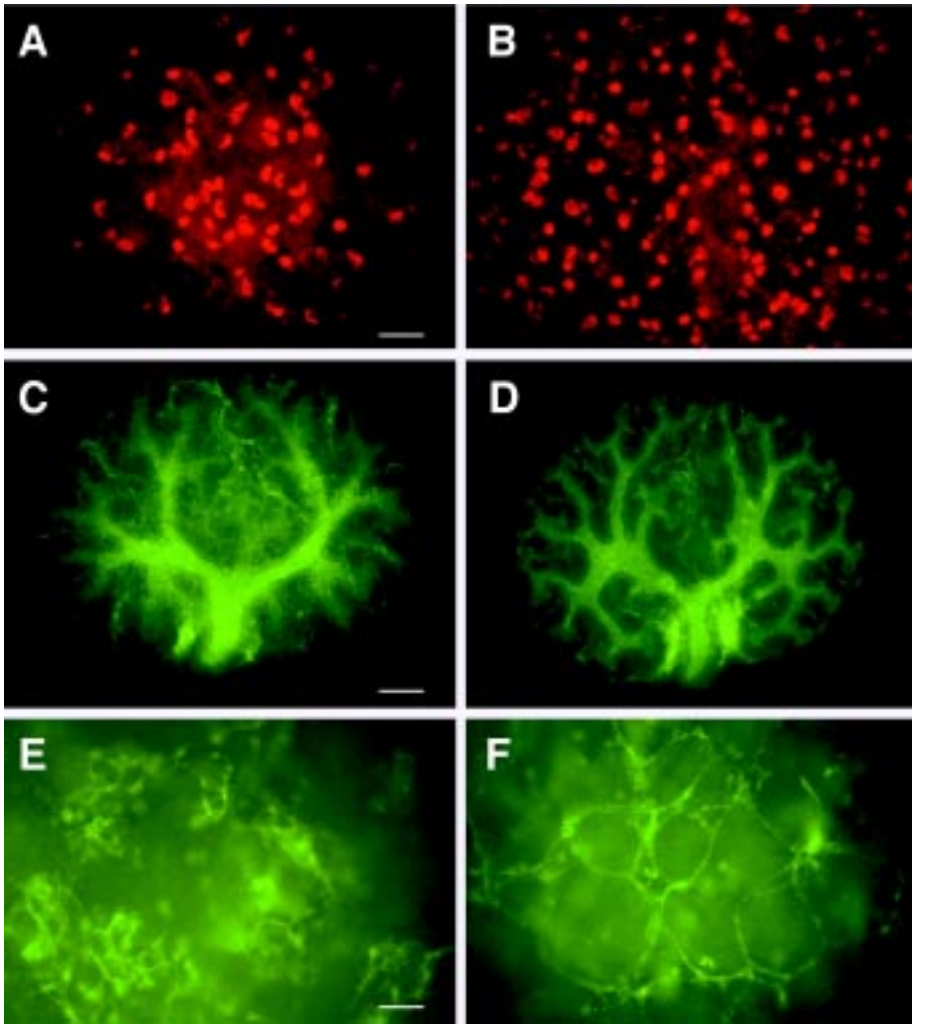

Fig. 1. Effect of retinoic acid on metanephros differentiation *in vitro* as assessed by lectin histochemistry. *Glomerular structures* **(A,B)**, *ureteric bud branches* **(C,D)**, *and vascular network* **(E,F)** *were visualized in whole metanephros organ cultures using Arachis hypogaea agglutinin, Dolichos biflorus agglutinin, and griffonia simplicifolia IB4, respectively. Metanephros were explanted from 14-day-old rat embryos and grown for 2* **(C,D)** *or 6 days* **(A,B,E,F)** *in defined medium with* **(B,D,F)** *or without* **(A,C,E)** *100 nM of retinoic acid. Both the number of glomeruli* **(B** *versus* **A)** *and of ureteric end buds* **(D** *versus* **C)** *were markedly increased. The architecture of vascular network is disorganized in the absence of retinoic acid* **(E)**, *but preserved in its presence* **(F)**. *Bar, 300 μm (A,B), 150 μm (C,D), and 100 μm (E,F).*

sufficient to induce supernumerary nephrons (Lelièvre-Pégorier *et al.*, 1998b). Lastly, giving retinol palmitate to protein-deprived pregnant rats was able to prevent the nephron deficit normally observed in the growth retarded pups (Lelièvre-Pégorier *et al.*, 1998c). These data together with the fact that low plasma vitamin A is a common feature of fetal growth retardation (Rondo *et al.*, 1995), suggest that the nephron deficit associated with intrauterine growth retardation results from a low vitamin A status.

The stimulating effect of retinoids on nephron number was found to result from an effect of retinoids upon ureteric bud branching capacity (Fig. 1C,D) (Vilar *et al.*, 1996). Growth and branching of the ureteric bud require glial cell line-derived neurotrophic factor (GDNF), a mesenchyme-derived secreted signal, and activation of its receptor-tyrosine kinase Ret which is present in the ureter tips (Sariola and Sainio, 1997). We found that expression of the *c-ret* gene is stimulated in a dose-dependent manner by adding retinoic acid to cultured metanephroi (Fig. 2), whereas *GDNF* gene expression is unaffected (Moreau *et al.*, 1998). Moreover, *c-ret* gene expression was low in metanephroi of 15-day-old vitamin A-deficient fetuses (Lelièvre-Pégorier *et al.*, 1998b).

Two recent reports indicate that retinoic acid also influences kidney vascular development. Kloth *et al.* (1998) used renal cortex explants prepared from the kidneys of newborn rabbits, and found that retinoic acid added to the culture medium preserved the regular spatial organization of the vascular network for 2 weeks. We found that retinoic acid is also involved in the control of blood vessel development in rat metanephric organ cultures during the early stages of renal organogenesis (Vilar *et al.*, 1998). The architecture of the vascular network survived for only two days in the metanephros taken from a 14-day-old fetus and cultured in serum free medium, without retinoic acid. The vascular network, however, survived for up to 6 days when retinoic acid was added to the culture medium (Fig. 1E,F).

Mild vitamin A deficiency *in utero* and programming of adult diseases

As in other models of inborn nephron deficit previously investigated, glomerular lesions are expected to develop in rats exposed *in utero* to vitamin A deficiency (see references in Merlet-Bénichou *et al.*, 1999a). Of interest is the fact that these rats also develop hypertension in adult life (Merlet-Bénichou *et al.*, 1999a). Given the role of vitamin A in renal organogenesis, the development of hypertension may result from the nephron deficit (Brenner *et al.*, 1988), but defects of renal vascular development may also occur and be involved in the etiology of hypertension. Intrauterine programming of hypertension has previously been reported to be associated with fetal growth retardation (Barker *et al.*, 1989). Because low circulating levels of vitamin A are also associated with fetal growth retardation (Rondo *et al.*, 1995), it is suggested that a lack of vitamin A is determinant in this programming. The recent observation from our group that no hypertension develops in growth retarded rats born to mothers deprived of proteins, but supplemented with vitamin A, supports this hypothesis (Merlet-Bénichou *et al.*, 1998).

Only large changes in vitamin A status have been so far considered to be a risk factor for the fetus. The above reviewed data allow us to speculate that mild vitamin A deficiency may cause nephron deficits and perhaps renal vascular defects, that are not recognized at birth, but may nevertheless induce long-term functional consequences. Although in industrialized countries vitamin

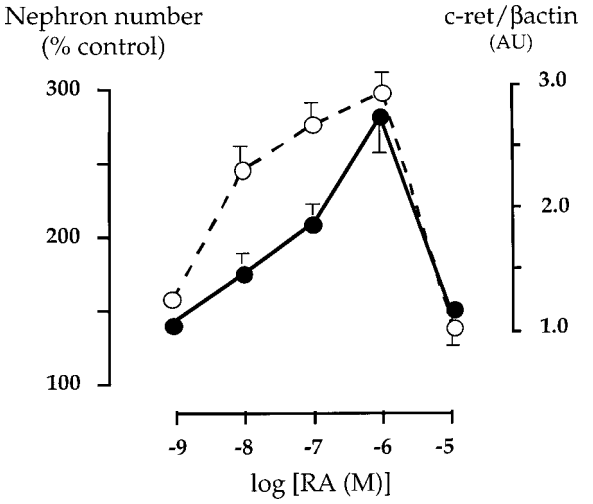

Fig. 2. Nephron number and c-ret expression as a function of retinoic acid concentration in metanephric organ culture. *The c-ret mRNA level (closed circles) after 4 days in culture depends on the retinoic acid concentration in the serum-free medium. The effect on c-ret expression predates the effect on the total number of nephrons (open circles) counted after six days in culture, which has a similar retinoic acid-response profile. Nephron numbers are expressed as the percentage of the corresponding paired control values. AU, arbitrary units; RA, retinoic acid.*

A deficiency is not a major health problem as it is in some developing countries, recent reports indicate that vitamin A intake and plasma retinol of the general population varies widely and may in some instances become insufficient (Gerster, 1997). Inadequate intakes occur due to dietary practices. Low circulating levels of pro-vitamin A or vitamin A are also encountered in several situations, the most common in women of child-bearing age being smoking, alcohol abuse, and uncontrolled weight-reducing diets. Finally, in a number of cases, low vitamin A stores resulting from inadequate intake are not sufficient to meet the increased demands encountered during pregnancy (see references in Merlet-Bénichou *et al.*, 1999b).

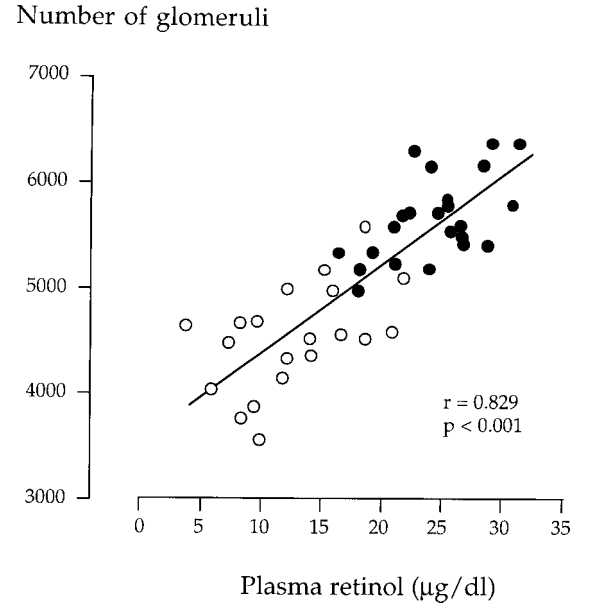

Fig. 3. Nephron number and plasma retinol in term rat fetuses. *The number of nephrons is closely correlated to plasma retinol in control (closed circles) and vitamin-A deficient (open circles) 21-day-old fetuses (r= 0.829, n= 44, p<0.001).*

Impaired nephrogenesis after hyperglycemia or drug exposure

Congenital malformations of various organs, including the kidney, occur with increased frequency in the offspring of diabetic mothers (Becerra *et al.*, 1990). Impaired renal organogenesis has been observed *in vitro* in the presence of elevated D-glucose concentrations (Kanwar *et al.*, 1996). Moderate hyperglycemia induced in unrestrained pregnant rats by continuous glucose perfusion over a four day period corresponding to early nephrogenesis was also found to cause a permanent nephron deficit in otherwise normal pups (Lelièvre-Pégorier *et al.*, 1998a).

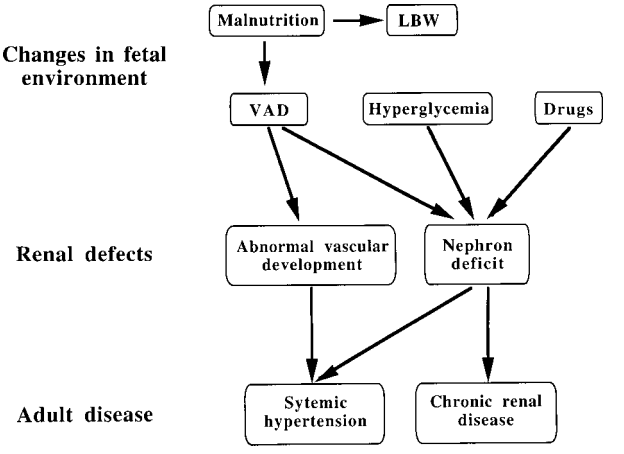

Fig. 4. Current hypotheses on the relationships between changes in fetal environment, renal defects and intrauterine programming of adult renal disease and systemic hypertension. *LBW, low birth weight; VAD, vitamin A deficiency.*

Interestingly, exposure to a diabetic intrauterine environment was recently shown to increase the risk of elevated urinary albumin excretion in diabetic Pima Indians (Nelson *et al.*, 1998), suggesting that these patients have *in utero*-acquired nephron deficit .

Studies conducted in animals have revealed that drugs prescribed during pregnancy and considered to be safe for the fetus may in fact impair kidney development (see references in Merlet-Bénichou *et al.*, 1999a). When given to pregnant rats, gentamicin, an antibiotic of the aminoglycoside group, reaches the fetal kidney where it causes subcellular lesions subsequently leading to a permanent nephron deficit. We used metanephros organ culture to reproduce the renal defect *in vitro*, and found that a defect in branching morphogenesis of the ureteric bud was the leading event of gentamicin-induced oligonephronia. More recently, we studied β-lactam antibiotics, which are among the most widely taken drugs during pregnancy (Gilbert *et al.*, 1997). Two amino-penicillins, ampicillin and amoxicillin, were found to reduce the number of nephrons both *in vitro* and *in vivo*, at a dosage normally used in clinical practice. No alteration of the ureteric bud branching pattern was found, but an increased amount of apoptotic figures

was observed in the induced mesenchyme. Besides the mild oligonephronia, tubular cystic dilatations were observed. By contrast, *in utero* exposure to ceftriaxone, a third generation cephalosporin, had no effect on the number of nephrons. Renal hypoplasia with cysts was recently reported in an infant who was exposed *in utero* to gentamicin and glucocorticoids (Hulton and Kaplan, 1995). No definite conclusion can be drawn, however, about the effect of gentamicin in this case, because glucocorticoids also alter nephrogenesis, at least in the rat (Celsi *et al*., 1998). Finally, we also found that cyclosporine A alters nephrogenesis *in vitro* (Merlet-Bénichou *et al*., 1999a). Nephron formation was arrested, possibly due to blockade of the conversion of metanephric mesenchyme to epithelium.

Conclusion

There is increasing evidence that the number of nephrons and perhaps other processes in renal organogenesis, such as vascular development, are influenced by changes in fetal environment (Fig. 4). The finding that the number of nephrons strictly depends on the circulating vitamin A level suggests that differences in vitamin A supply to the fetus can account for the wide range of nephron numbers in humans. Other exogenous factors, such as maternal hyperglycemia or drugs given during pregnancy were also found to alter nephrogenesis in animals. Although they may not influence the number of nephrons to the same extent as vitamin A, they might contribute to some cases of nephron deficits. As previously suggested for infants with low birth weight, whose renal defects may also result from low vitamin A levels, a number of apparently normal babies exposed *in utero* to mild vitamin A deficiency, maternal diabetes mellitus or drugs, are at risk of developing chronic renal disease and systemic hypertension later in life. Clinical confirmation of this hypothesis is needed.

References

BARKER, D.J.P., OSMOND, C., GOLDING, J., KUH, D. and WADSWORTH, M.E.J. (1989). Growth *in utero,* blood pressure in childhood and adult life, and mortality from cardiovascular disease. *Br. Med. J. 298:* 564-567.

BECERRA, J., KHOURY, M., CORDERO, J. and ERIKSON, J. (1990). Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. *Pediatrics 85:* 1-9.

BRENNER, B. and CHERTOW, G. (1993). Congenital oligonephropathy: An inborn cause of adult hypertension and progressive renal injury? *Curr. Opin. Nephrol. Hypertens. 2:* 691-695.

BRENNER, B.M., GARCIA, D.L. and ANDERSON, S. (1988). Glomeruli and blood pressure. Less of one, more of the other? *Am. J. Hypertens. 1:* 335-347.

CELSI, G., KISTNER, A., AIZMAN, R., EKLOF, A., CECCATELLI, S., DE SANTIAGO, A. and JACOBSON, S. (1998). Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. *Pediatr. Res. 44:* 317-322.

GERSTER, H. (1997). Vitamin A - Functions, dietary requirements and safety in humans. *Int. J. Vitam. Nutr. Res. 67:* 71-90.

GILBERT, T., NATHANSON, S., MOREAU, E., VILAR, J., LELIÈVRE-PÉGORIER, M. and MERLET-BÉNICHOU, C. (1997). Penicillins and cephalosporins impair nephrogenesis *in vitro. J. Am. Soc. Nephrol. 8:* 359 (Abstr.).

HULTON, S.A. and KAPLAN, B.S. (1995). Renal dysplasia associated with *in utero* exposure to gentamicin and corticosteroids. *Am. J. Med. Genet. 58:* 91-93.

KANWAR, Y., LIU, Z., KUMAR, A., USMAN, M., WADA, J. and WALLNER, E. (1996). D-glucose-induced dysmorphogenesis of embryonic kidney. *J. Clin. Invest. 98:* 2478-2488.

KLOTH, S., GERDES, J., WANKE, C. and MINUTH, W.W. (1998). Basic fibroblast growth factor is a morphogenic modulator in kidney vessel development. *Kidney. Int. 53:* 970-978.

LELIÈVRE-PÉGORIER, M., AMRI, K., FREUND, N., MOREAU, E., VILAR, J., GILBERT, T. and MERLET-BÉNICHOU, C. (1998a). Mild hyperglycemia induces nephron deficit *in vivo* and *in vitro* in the rat. *J. Am Soc. Nephrol. 9:* 501 (Abstr.).

LELIÈVRE-PÉGORIER, M., VILAR, J., FERRIER, M., MOREAU, E., FREUND, N., GILBERT, T. and MERLET-BÉNICHOU, C. (1998b). Mild Vitamin A deficiency leads to inborn nephron deficit in the rat. *Kidney Int. 54:* 1455-1462.

LELIÈVRE-PÉGORIER, M., VILAR, J., FREUND, N., GILBERT, T. and MERLET-BÉNICHOU, C. (1998c). Vitamin A prevents nephron deficit in growth-retarded fetal rats. *J. Am. Soc. Nephrol. 9:* 501 (Abstr.).

MENDELSOHN, C., LOHNES, D., DÉCIMO, D., LUFKIN, T., LEMEUR, M., CHAMBON, P. and MARK, M. (1994). Function of the retinoic acid receptors (RARs) during development. (II) Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development 120:* 2749-2771.

MERLET-BÉNICHOU, C., GILBERT, T., VILAR, J., MOREAU, E., FREUND, N. and LELIÈVRE-PÉGORIER, M.(1999a). Nephron number: variability is the rule. Causes and consequences.*Lab. Invest. 79:* 515-527.

MERLET-BÉNICHOU, C., VILAR, J., GILBERT, T. and LELIÈVRE-PÉGORIER, M. (1998). Need for new hypothesis in the fetal origin of hypertension. Is vitamin A involved? *J. Am. Soc. Nephrol. 9:* 312 (Abstr.).

MERLET-BÉNICHOU, C., VILAR, J., LELIÈVRE-PÉGORIER, M. and GILBERT, T. (1999b). Role of retinoids in renal development : pathophysiological implication. *Curr. Opin. Nephrol. Hypertens. 8:* 39-43.

MOREAU, E., VILAR, J., LELIÈVRE-PÉGORIER, M., MERLET-BÉNICHOU, C. and GILBERT, T. (1998). Regulation of c-ret expression by retinoic acid in rat metanephros: implication in nephron mass control. *Am. J. Physiol. 275:* F938-F945.

NELSON, R., MORGENSTERN, H. and BENNETT, P. (1998). Intrauterine diabetes exposure and the risk of renal disease in diabetic Pima Indians. *Diabetes 47:* 1489-1493.

RONDO, P.H.C., ABBOT, R., RODRIGUES, L.C. and TOMKINS, A.M. (1995). Vitamin A, folate, and iron concentrations in cord and maternal blood of intra-urine growth retarded and appropriate birth weight babies. *Eur. J. Clin. Nutr. 49:* 391-399.

SARIOLA, H. and SAINIO, K. (1997). The tip-top branching ureter. *Curr. Opin. Cell. Biol. 9:* 877-884.

SAXÉN, L. (1987). *Organogenesis of the kidney*, (eds. P. Barlow, P. Green and C. Wylie). Cambridge University Press, Cambridge.

TELLIER, A.-L., AMIEL, J., SALOMON, R., JOLLY, D., DELEZOIDE, A.-L., AUGE, J., GUBLER, M.-C., MUNNICH, A., LYONNET, S., ANTIGNAC, C., VEKEMANS, M., BROYER, M. and ATTIE-BITACH, T. (1998). PAX2 expression during early human development and its mutations in renal hypoplasia with or without coloboma. *Am. J. Hum. Genet. 63 (Suppl.):* A7 (Abstr.).

VILAR, J., GILBERT, T., MOREAU, E. and MERLET-BÉNICHOU, C. (1996). Metanephros organogenesis is highly stimulated by vitamin A derivatives in organ culture. *Kidney Int. 49:* 1478-1487.

VILAR, J., NAUD, N., LELIÈVRE-PÉGORIER, M., GILBERT, T. and MERLET-BÉNICHOU, C. (1998). Retinoic acid is required for the early organization of renal vascular network. *J. Am. Soc. Nephrol. 9:* 369 (Abstr.).

WILSON, J.G. and WARKANY, J. (1948). Malformations in the genito-urinary tract induced by maternal vitamin A deficiency in the rat. *Am. J. Anat. 83:* 357-407.