Iyanda, 1:1
http://dx.doi.org/10.4172/scientificreports.137

Open Access Scientific Reports

Research Article Open Access

Serum Vitamin Levels in Different Categories of Androgenetic Alopecia Subjects

Ayobola Abolape Iyanda*

Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola, University of Technology, Osogbo, Nigeria

Abstract

Introduction: Environmental factors such as cigarette smoke and alcohol alter vitamin levels and both have been linked with androgenetic alopecia (AGA). The purpose of this study is to determine the levels of serum vitamins in AGA subjects and observe if altered vitamin levels play a role in the pathogenesis of alopecia in smokers and alcohol consuming subjects.

Methods: Using the high performance liquid chromatographic technique, serum vitamin levels were determined and data were obtained on durations of alopecia, smoking and alcohol consumption. A possible relationship between these parameters was determined using Pearson's correlation coefficient.

Results: The levels of serum vitamins in non-smoking/non-alcohol consuming AGA subjects were not significantly different ($p>0.05$) compared with control although those of smoking and alcohol consuming subjects were significantly lower ($p<0.05$), especially vitamins A, C & E and niacin (antioxidant vitamins). Correlation study did establish a relationship between durations of alopecia and smoking but not alcohol consumption, and alopecia commenced much earlier in both smoking and alcohol consuming AGA subjects than non-smoking/non-alcohol consuming alopecia subjects.

Conclusion: Although there may not be any relationship between serum vitamin levels and duration of alopecia, both smoking and alcohol seem to be likely factors that can trigger AGA much early in subjects who have susceptibility for it.

Keywords: Serum vitamins; Smoking; Alcohol; Androgenetic alopecia

Introduction

Sawant et al. [1] have described hair loss as a universal problem. Androgenetic alopecia (AGA) frequently identified as male pattern baldness affects up to 50% of men worldwide [2]. The disorder occurs in many patients below 40 years of age and in some patients below the age of 30 years [3]. It occurs in both male and female subjects but is more commonly found in males. It is a condition characterized by loss of hair from the scalp in a defined pattern. Some of the factors known to determine an individual's tendency to manifest AGA include genetic predisposition coupled with the presence of sufficient circulating androgens. Among the Caucasians the prevalence of this condition is greater than 50% especially among males 50 years and above. Although in many subjects no serious direct health consequences (metabolic) may occur as a result of this condition but loss of scalp hair is quite distressing.

Psychological impacts of this condition include low self-esteem and loss of self-confidence [4]. Hair loss has been reported to affect personal attractiveness and social life [5], these may be the cause of the low self-esteem and loss of self-confidence associated with hair loss; a source of significant distress among men [4]. A study by Cash et al. [6] carried out on both sexes has also confirmed that AGA is a stressful condition affecting the psychological functioning of an individual [6]. Although in many developing countries androgenetic alopecia when it is not accompanied with abnormal health state, is viewed as a cosmetic problem especially in males, yet its psychological impacts is not diminished. It is viewed more as a cosmetic problem probably because of an array of other more pressing problems (e.g. socioeconomic) confronting the males affected by this disorder.

The pathogenesis of androgenetic alopecia though has not been fully elucidated, yet studies have shown that transformation takes place in the pre-programmed follicles on the scalp such that these follicles feature a change from long growth (anagen) and short rest (telogen) cycles, to long rest and short growth cycles, as well as progressive miniaturisation of the follicle result in hair loss [7]. Although a role for androgen has been identified in both processes, requirement of inheritance of several genes has also been highlighted as essential ingredients in the pathogenesis of AGA. One of the genes which has been identified and closely linked with AGA is that which encodes the androgen receptor (AR) [7].

But many other studies have confirmed that the phenomenon of unexplained hair loss is multifactorial. A number of biochemical compounds and elements have been associated with hair loss. Deficiencies of essential fatty acids result in alopecia, others causes are zinc deficiency and biotin deficiency. Depletion in levels of iron and ferritin is also a common cause of hair loss occurring as a result of long-term parenteral nutrition [8]. Biotin deficiency though is a very rare cause of hair loss [9]. Vitamin D deficiency has also been associated with diffuse hair loss [10, 11], probably due to its role as essential vitamin in cell growth.

Inadequate nutritional status irrespective of cause [12] has been

***Corresponding author:** Ayobola Abolape Iyanda, Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola, University of Technology, Osogbo, Nigeria; Phone No +2347039407465; E-mail: lapeiyanda@yahoo.com

Received March 27, 2012; **Published** July 06, 2012

Citation: Iyanda AA (2012) Serum Vitamin Levels in Different Categories of Androgenetic Alopecia Subjects. 1: 137. doi:10.4172/scientificreports.137

linked with alopecia and only a few vitamins have been investigated in alopecia patients, the aim of this study is to determine the serum levels of a wide range of vitamins, especially many of which had not hitherto been monitored in androgenetic alopecia subjects in the Nigerian environment. This is because many vitamins have been linked with hair development and are also known for their antioxidant roles. Oxidative stress has been linked with a number of clinical conditions.

Materials and Methods

Study site

It is a cross-sectional study and because many males considered AGA as a cosmetic problem rather than a medical one, the subjects were recruited randomly out of willing participants residing in Ibadan metropolis.

Study subjects

The male subjects with male pattern hair loss selected for the study were identified using Norwood classification of hair loss. Males with female pattern alopecia were not recruited for the study. Thirty subjects were randomly selected to constitute each of the alopecia groups while 40 served as the control. Information was obtained from each subject about age, age at onset of AGA, number of cigarette smoked and quantity of alcohol consumed if any. From each smoker, information on age at commencement of smoking; and quantity of cigarette smoked per day, duration, and frequency of smoking were also obtained. Moreover, subjects in alcohol group supplied information on age at commencement of alcohol consumption; average quantity of alcohol consumed per day, duration, and frequency of alcohol consumption.

Ethics

All study procedures conformed to the principle outlined in the Declaration of Helsinki in 1975 (revised in 2000). The male subjects satisfying the inclusion and exclusion criteria were recruited for the study.

Inclusion criteria for both alopecia and control subjects

Male subjects presenting with AGA having grade III to VI (according to Norwood classification of hair loss) and from the age of above 20 to 60 years were recruited for the study. They were divided into three groups based on their exposure to two environmental factors which past studies have identified to play a role in the pathogenesis of androgenetic alopecia; namely alcohol and cigarette smoke.

Group A: male alopecia subjects who are smokers (currently smoking an average of 10 cigarette sticks per day).

Group B: male alopecia subjects who take on average at least approximately 2 drinks a day.

Group C: male alopecia subjects not exposed to cigarette smoke and alcohol consumption.

Group D: male non-alopecia subjects not exposed to cigarette smoke and alcohol consumption.

Exclusion criteria

Subjects with any other type of alopecia

Family history of any other type of alopecia

Physiological or emotional stress

History of medical conditions capable of alopecia

Nutritional causes e.g. starvation, malnutrition, malabsorption

Medication capable of causing alopecia e.g. angiotensin-converting enzyme inhibitors, anticonvulsants, antidepressants, and the anticoagulants heparin and warfarin)

Vitamin supplementation

Serum vitamin estimation

Ten ml of blood was obtained from anti cubital vein of each subject between the hours of 9.00 and 12.00. A standard blood collection regimen was established for all subjects. Blood samples were centrifuged for 10 minutes at 3000 r.p.m. to obtain sera which were stored at -20°C until utilized for serum vitamin estimation. The serum concentrations of the following vitamins (folic acid, riboflavin, niacin, vitamins A, C, D & E) were determined. Vitamin levels were estimated using the high performance liquid chromatographic techniques (HPLC). Waters 626 LC SYSTEM was used for this purpose.

Statistical analysis

The degree of significant difference between each of the alopecia group and the control was determined using Student't' test. Inter-group comparison was achieved using analysis of variance (ANOVA). The SPSS package (version 15) was used for the statistical analysis. The degree of correlation between the vitamins and other variables was established using Pearson's correlation coefficient. P value of < 0.05 was considered significant in each case.

Results

Table 1 shows significant decreases in the serum levels of niacin, folic acid, vitamins A, C and E ($p \leq 0.05$) in the smoker group when compared with control, using Student t test. In the same category of subjects, riboflavin and vitamin D were not significantly different ($p \geq 0.05$). Inter-group comparison using ANOVA confirmed the significant differences. Also in group 1 none of the vitamins was significantly different in groups B and C ($p \leq 0.05$). Results in Table 2 show that vitamins A, C and E as well as niacin were significantly decreased ($p \leq 0.05$) while vitamin D was not significant different ($p \geq 0.05$) in alcohol consuming group compared with control.

	GROUP A Mean ± SEM	GROUP C Mean ± SEM	GROUP D Mean ± SEM	F values	P values
Vitamin A (µmol/L)	1.32 ± 0.04*	1.55 ± 0.05	1.57 ± 0.05	9.253	0.004†
Vitamin E (µmol/L)	19.37 ± 1.17*	25.00 ± 1.37	24.73 ± 1.16	6.596	0.002†
Vitamin C (µmol/L)	43.93 ± 3.36*	64.47 ± 3.54	63.83 ± 3.60	11.119	0.003†
Vitamin D (nmol/L)	113.57 ± 4.68	112.60 ± 5.27	108.83 ± 5.19	0.245	0.783
Folic acid (nmol/L)	14.83 ± 0.85*	18.20 ± 0.86	17.67 ± 0.79	4.744	0.011†
Niacin (nmol/L)	20.67 ± 1.71*	29.90 ± 1.79	30.30 ± 1.89	9.166	0.001†
Riboflavin (µmol/L)	151.53±4.41	149.17±5.51	151.17±5.51	0.004	0.996

*Mean±SEM- mean±standard error of mean. GROUP A- androgenetic alopecia subject exposed to cigarette smoke; GROUP C- non-smoking/non-alcohol androgenetic alopecia subjects; GROUP D- control subjects. * Significant at $p \leq 0.05$ using Student t test; † Significant at $p \leq 0.05$ using ANOVA.

Table 1: Serum levels of selected vitamins of smoking; non-smoking/non-alcohol consuming androgenetic alopecia subjects and controls.

	GROUP A Mean ± SEM	GROUP C Mean ± SEM	GROUP D Mean ± SEM	F values	P values
Vitamin A (µmol/L)	1.37 ± 0.05*	1.55 ± 0.05	1.57 ± 0.05	5.578	0.005†
Vitamin E (µmol/L)	31.10 ± 1.67*	25.00 ± 1.37	24.73 ± 1.16	6.442	0.002†
Vitamin C (µmol/L)	51.13 ± 2.01*	64.47 ± 3.54	63.83 ± 3.60	5.738	0.005†
Vitamin D (nmol/L)	142.97 ± 5.10*	112.60 ±5.27	108.83 ± 5.19	13.000	0.001†
Folic acid (nmol/L)	18.33 ± 0.91	18.20 ± 0.86	17.67 ± 0.79	0.171	0.843
Niacin (nmol/L)	23.03 ± 1.62*	29.90 ± 1.79	30.30 ± 1.89	5.320	0.007†
Riboflavin (µmol/L)	154.37 ± 4.73	149.17 ± 5.51	151.17 ± 5.51	0.106	0.899

Mean±SEM- mean±standard error of mean GROUP B- androgenetic alopecia subject consuming alcohol; GROUP C- non-smoking/non-alcohol consuming androgenetic alopecia subjects; GROUP D- control subjects. * Significant at p ≤0.05 using Student t test; † Significant at p ≤0.05 using ANOVA.

Table 2: Serum levels of selected vitamins of alcohol consuming, non-smoking/non-alcohol consuming androgenetic alopecia and controls.

The results of the correlation study between duration of exposure and vitamins on one hand and duration of alopecia and duration of smoking/alcohol consumption on the other hand show that there was no correlation between the variables, except vitamin D that was negatively correlated with duration of alopecia (r = -0.365, p = 0.048).

Discussion and Conclusion

According to Stenn and Paus [13] hair growth cycle which is controlled by a chemical signal like epidermal growth factor can be disrupted in alopecia and this disruption has been linked to abnormality in the levels of a number of biomolecules. Although extensive work has been done in AGA subjects in relation to other biomolecules like testosterone and other micronutrients e.g. trace elements, fewer studies exist which have addressed a possible link between androgenetic alopecia and serum vitamin levels. Statistical analysis of the results of this study, using ANOVA shows that a number of vitamins are significantly different as revealed in Tables 1 & 2; but these differences can be ascribed to the effect of cigarette smoke and alcohol consumption since the results of the non-smoking/non-alcohol consuming group did not manifest such differences, when non-smoking/non-alcohol consuming group was compared with control using Student t test. This is not surprising since both smoking and alcohol consumption induce oxidative stress and many of these vitamins possess antioxidant properties.

Vitamins A, C and E which are known to constitute significant antioxidant potential of the plasma [14] were found to be significantly reduced in both smoking and alcohol consuming groups. That these significant differences in the cigarette smoking and alcohol consuming group may not be directly related to alopecia is evident from the result of correlation study; that shows that there was no correlation between duration of alopecia and most of all the vitamins in all groups. Moreover, although the mean age of the subjects in the three categories of alopecia are comparable and therefore not significantly different, the age of commencement of alopecia was found to be much higher in non-smoking/non-alcohol consuming group than other two groups which suggests that oxidative stress-induced mechanisms set in motion by these xenobiotics are capable of causing early onset of this condition in subjects who have susceptibility for it.

The findings of normal serum vitamin levels observed in non-smoking/non-alcohol consuming alopecia subjects may be an indication that alteration in vitamin levels is not a common occurrence in this condition. Vitamin D and biotin deficiencies which hitherto had been identified to be associated with alopecia [11] were not significantly different in alopecia groups compared with controls, although studies have suggested that vitamin D plays a role in the proliferation and differentiation of keratinocytes [15]. Serum vitamin E level, that its daily supplements has been reported to function in increasing hair growth in people with male pattern alopecia was also not significantly different in the non-smoking/non-alcohol consuming alopecia group compared with control.

Moreover, the results of this study recorded non-significant differences for both serum vitamins A and C in non-smoking/non-alcohol consuming alopecia group compared with controls. Even though studies have established that both zinc and selenium help in the proper utilization of proteins and hormones in hair formation, and vitamins A, C, E and vitamin B6 in conjunction with these two minerals are important in promoting hair regrowth. Durusoy et al. [16], have also indicated that no evidence exist that serum levels of zinc, folate or vitamin B(12) are involved in the pathogenesis of trichodynia in patients with diffuse alopecia. Their findings are in consonance with our result, in which non-significant differences were recorded in the serum levels of folate and vitamin C. This is despite the fact that prior to 1980 substances such as ascorbic acid, benzoic acid, B vitamins, hormones, jojoba oil, lanolin etc. were commonly used in the United States of America for the treatment of hair loss.

Although serum levels of most of these vitamins are not significantly different from those of control in subjects not exposed to both cigarette smoke and alcohol, this does not exclude their low levels or those of their derivatives in the intracellular compartments of hair follicle where they may be required for normal biochemical processes necessary for

		Duration of alopecia	Duration of smoking	Duration of alcohol consumption
Vitamin A	GROUP A	r = -0.237; p = 0.207	r = -0.199; p = 0.297	r = -0.138;p= 0.466
	GROUP B	r = 0.142; p = 0.454		
	GROUP C	r = 0.163; p = 0.389		
Vitamin C	GROUP A	r = 0.010; p=0.960	r = 0.079; p = 0.680	r = 0.095; p = 0.617
	GROUP B	r = 0.015; p = 0.939		
	GROUP C	r = -0.095; p = 0.614		
Vitamin E	GROUP A	r = 0.011; p = 0.954	r = 0.040; p = 0.832	r = 0.163; p = 0.389
	GROUP B	r = -0.019; p = 0.922		
	GROUP C	r = -0.184; p = 0.330		
Vitamin D	GROUP A	r = -0.365; p = 0.048*	r = -0.173; p = 0.361	r = -0.045; p = 0.812
	GROUP B	r = 0.070; p = 0.715		
	GROUP C	r = 0.355; p = 0.054		
Folic	GROUP A	r = -0.112; p = 0.555	r = 0.092; p= 0.630	r = -0.333; p= 0.072
	GROUP B	r = -0.161; p = 0.395		
	GROUP C	r = -0.040; p = 0.835		
Niacin	GROUP A	r = 0.024; p = 0.898	r = 0.062; p = 0.743	r = 0.009; p = 0.962
	GROUP B	r = 0.064; p = 0.737		
	GROUP C	r = -0.060; p = 0.751		
Riboflavin	GROUP A	r = 0.149; p = 0.433	r = 0.261; p = 0.164	r = -0.008; p = 0.968
	GROUP B	r = 0.024; p = 0.899		
	GROUP C	r = -0.221; p = 0.240		

Level of significance is set at p≤0.05. GROUPS A, B, C are smoking, alcohol consuming, non-smoking/non-alcohol consuming alopecia subjects. * Significant at p ≤0.05.

Table 3: Correlation between durations of exposure, smoking and alcohol consumption and serum vitamin levels in three categories of alopecia subjects.

hair morphogenesis. Manganese has been reported to be low in hair but normal in serum [17], a situation which could have occurred as a result of abnormal cellular uptake by hair follicle.

Moreover, since one of these vitamins that have been reported to be involved in hair formation (vitamin D) is a lipid-hormone and lipid-soluble hormone such as vitamin D is transported in plasma bound to carrier protein with a small quantity of the hormone being in the unbound form. The free/unbound form enters the cells by passive diffusion and thereby binds to the intracellular receptors of the cytoplasm or nucleus, thus causing alteration in the molecular conformation of the intracellular receptor. This alteration is what is responsible for the binding of hormone-receptor complex to specific regulatory DNA sequences of some target genes, in this case, gene which may be involved with hair formation. In addition, since receptor problem in the presence of a normal serum vitamin levels has been recognized as being the basis of many metabolic diseases e.g. vitamin D resistant rickets [18,19], abnormal cell receptor may be considered in these categories of subjects. To further support such possibility is the fact that abnormal vitamin D receptor of the hair follicle is a known cause of some types of alopecia.

Hair follicles are unique structures that are capable of exceptional regenerative potential. They are essential for epidermal homeostasis and reepithelialization after damage to human skin. Like other, more active and quickly proliferating organ systems, hair follicles may be easily disturbed in their normal growth cycle by systemic and local influences. An example of such influences is that of dehydrotestosterone (DHT), a molecule obtained from the action of enzyme 5-alpha reductase (both types I and II) on testosterone. DHT among other things causes damage to hair follicles, leading to the inflammatory reaction to the scalp and progressive miniaturization of hair follicles [20]. This may be the route by which abnormality of the receptor can arise. That receptor problem is a possibility in these subjects is the fact that many subjects that use substances that reduce the level of DHT still have alopecia, this probably points to the involvement/abnormality of other biochemical components apart from testosterone and its derivatives in the pathogenesis of alopecia.

Androgenetic alopecia causes loss of self-esteem, depression, introversion, neuroticism and feeling of unattractiveness which have been identified in several categories of subjects. Since current available treatment modalities with proven efficacy are oral finasteride (a competitive inhibitor of type 2 5 alpha-reductase), and topical minoxidil (an adenosine-triphosphate-sensitive potassium channel opener which has been reported to stimulate the production of vascular endothelial growth factor in cultured dermal papilla cells), have so far been associated with limited success [21]. Further studies on intracellular uptake and contents of some micronutrients of the hair or the hair follicle may be helpful, especially those vitamins/micronutrients that studies have shown to improve hair regrowth.

In addition, vitamin D receptor (VDR) independent of vitamin D has been shown to be important in hair cycling, specifically anagen initiation phase [22]. Studies have also demonstrated that absence of VDR leads to the development of alopecia and that the hair follicle is formed by reciprocal interactions between an epidermal placode, which gives rise to the hair follicle keratinocytes and the underlying mesoderm which gives rise to the dermal papilla [15]. Moreover, VDR null mice were unable to initiate a new hair cycle after the period of morphogenesis is complete and investigations in transgenic mice have demonstrated that restricted expression of the VDR to keratinocytes is capable of preventing alopecia in the VDR null mice, thus demonstrating that the epidermal component of the hair follicle requires VDR expression to maintain normal hair follicle homeostasis. In addition to the impact of VDR on epidermal differentiation, VDR has been shown to be essential for hair follicle integrity, such that humans with mutations in the VDR develop alopecia totalis [23]. Therefore there may also be the need to determine in male androgenetic alopecia subjects, vitamin D receptor activity and if possible to assess vitamin uptake by hair follicle, since topical application of vitamin-containing creams have been reported to favor hair growth.

References

1. Sawant N, Chikhalkar S, Mehta V, Ravi M, Madke B, et al. (2010) Androgenetic Alopecia: Quality-of-life and Associated Lifestyle Patterns. Int J Trichology 2: 81-85.
2. Thomas J (2005) Androgenetic Alopecia - Current status. Indian J Dermatol 50: 179-190.
3. Norwood OT (1975) Male pattern baldness: Classification and incidence. South Med J 68: 1359-1365.
4. Williamson D, Gonzalez M, Finlay AY (2001) The effect of hair loss on quality of life. J Eur Acad Dermatol Venereol 15: 137-139.
5. Alfonso M, Richter-Appelt H, Tosti A, Viera MS, García M (2005) The psychosocial impact of hair loss among men: A multinational European study. Curr Med Res Opin 21: 1829-1836.
6. Cash TF, Price VH, Savin RC (1993) Psychological effects of androgenetic alopecia on women: Comparisons with balding men and with female control subjects. J Am Acad Dermatol 29: 568-575.
7. Ellis JA, Sinclair R, Harrap SB (2002) Androgenetic alopecia: pathogenesis and potential for therapy. Expert Rev Mol Med 4: 1-11.
8. Daniells S, Hardy G (2010) Hair loss in long-term or home parenteral nutrition: are micronutrient deficiencies to blame? Curr Opin Clin Nutr Metab Care 13: 690-697.
9. Olsen EA, Fiedler VC, Gray AC (2003) Diffuse alopecia: telogen hair loss. In: Olsen EA, ed. Disorders of Hair Growth: Diagnosis and Treatment. (2nd ed) McGraw-Hill Publishing, New York, NY.
10. Bergfeld WF, Mulinari-Brenner F (2001) Shedding: how to manage a common cause of hair loss. Cleve Clin J Med 68: 256-261.
11. McMichael J, Hordin MK, Bergfeld WF (2008) Telogen effluvium. In: McMichael J, Hordin MK, eds. Hair and Scalp Diseases: Medical, Surgical, and Cosmetic Treatments. Informa Health Care, London, UK.
12. Rushton DH (2002) Nutritional factors and hair loss. Clin Exp Dermatol 27: 396-404.
13. Stenn KS, Paus R (2001) Controls of Hair Follicle Cycling. Physiol Rev 81: 449-494.
14. Shenkin A, Baines M, Fell G, Lyon TDG (2006) Vitamins and trace elements. In: Burtis C. A, Ashwood ER, Bruns D. E.(eds) Tietz textbook of Clinical Chemistry and Molecular Diagnostics. Saunders, Missouri.
15. Demay MB, MacDonald PN, Skorija K, Dowd DR, Cianferrotti L, Cox M (2007) Role of the vitamin D receptor in hair follicle biology. J Steroid Biochem Mol Biol 103: 344-346.
16. Durusoy C, Ozenli Y, Adiguzel A, Budakoglu IY, Tugal O, et al. (2009) The role of psychological factors and serum zinc, folate and vitamin B12 levels in the aetiology of trichodynia: a case-control study. Clin Exp Dermatol 34: 789-92.
17. Jin W, Zhu Z, Wu S, Zhang X, Zhou X (1998) Determination of zinc, copper, iron and manganese contents in hair for MPA patients and healthy men. Guang Pu Xue Yu Guang Pu Fen Xi 18: 91-93.
18. Welch TR, Bergstrom WH, Tsang RC (2000) Vitamin D-deficient rickets: the reemergence of a once-conquered disease. J Pediatr 137: 143-145.
19. Kreiter SR, Schwartz RP, Kirkman HN, Charlton PA, Calikoglu AS, et al. (2000) Nutritional rickets in African American breast-fed infants. J Pediatr 137: 153-157.

20. Sinclair R (1998) Male pattern androgenetic alopecia. BMJ 317: 865-869.

21. Hoffmann R, Happle R (2000) Current understanding of androgenetic alopecia. Part II: clinical aspects and treatment. European J Dermatol 10: 410-417.

22. Amor KT, Rashid RM, Mirmirani P (2010) Does D matter? The role of vitamin D in hair disorders and hair follicle cycling. Dermatol Online J 16: 3.

23. Malloy PJ, Hochberg Z, Tiosano D, Pike JW, Hughes MR, et al. (1990) The molecular basis of hereditary 1,25-dihydroxyvitamin D3 resistant rickets in seven related families. J Clin Invest 86: 2071-2079.