Inhibin and premature ovarian failure

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Introduction

- Premature ovarian failure (POF) is characterized by ovarian dysfunction leading to a menopause-like state < 40 years of age.
  - Prevalence:
    - 1% of all women < 40 years old
    - 0.1% in women 30 years or younger
  - Primary amenorrhea: not experience a menstrual cycle at all
  - Secondary amenorrhea: experience cessation of ovarian function after a period of menstrual cycling

Introduction

- Dx criteria of POF:
  - ↑ serum FSH levels (> 40 IU/l)
  - Amenorrhoea for duration of > 6 months or
  - Very low levels of circulating estrogen

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- Clinical symptoms of POF:
  - Menopause symptoms like hot flushes, vaginal dryness, dyspareunia, insomnia, vaginitis and mood swings
  - Psychological disturbance including depression
  - ↑ risk for CVD
  - Low bone density (osteoporosis)
  - 2-fold age-specific ↑ in mortality rate

Introduction

- The etiology of POF:
  - heterogeneous with the majority being idiopathic
  - Known causes:
    - permanent damage to the ovaries: pelvic surgery, chemotherapy or radiotherapy, autoimmune conditions, exposure to environmental toxicants and genetic causes

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- Genetic abnormalities on the X chromosome regions Xq13.3– q21.3 and Xq26–28 give rise to the POF phenotype.
- POF related:
  - Fragile X and Turner's syndrome
  - Genes located on the X chromosome include DIAPH2, FSHPRH1 and LRPR1, BMP15 and ZFX.
  - Autosomal mutations in a # of dominantly inherited genes including GDF9, INHA, FOXL2, FSHR and others.

Introduction

- Inhibin:
  - as an endocrine modulator of pituitary FSH synthesis
  - act locally in the ovary, the most clearly defined paracrine function being to stimulate androgen biosynthesis in the theca cells
  - Other paracrine roles within granulosa cells include antagonism of activin, bone morphogenetic proteins (BMPs-2, -6 and -7).

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- Inhibin:
  - Inhibins and activins are members of the transforming growth factor β (TGFβ) superfamily encompassing a vast array of growth and differentiation factors such as TGFβs and BMPs.
  - inhibins regulate FSH secretion by inhibiting the stimulatory actions of the structurally related proteins, activins.

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- Inhibin:
  - heterodimers of an 18 kDa a-subunit disulphide-linked to one of two 14 kDa β-subunits (βA and βB)
  - inhibin A and B are expressed in the ovary and are secreted throughout the menstrual cycle in a discordant pattern
    - smaller follicles expressing mainly the α- and βB-subunits
    - dominant follicles and the corpus luteum express α- and βA-subunits.
- Activins are composed of 2 β-subunits; βA- βA (activin A), βA- βB (activin AB), βB- βB (activin B).

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- In early follicular phase, with ↑ FSH levels, there is an ↑ of plasma levels of inhibins with the ↑ in inhibin B more pronounced than inhibin A levels.
  - Inhibin B is mainly produced by the granulosa cells of the developing follicles, levels being highest in mid-follicular phase.
  - The dominant follicle selectively produces inhibin A its levels high mid-cycle leading to the suppression of FSH.
- In the luteal phase, the corpus luteum maintains

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- When the ovarian follicle reserve is depleted as a woman is approaching menopause, a ↓ in inhibin levels is correlated with ↑ pituitary FSH secretion.
- Luteal phase inhibin A and follicular phase inhibin B are correlated inversely with age in perimenopausal women and follicular phase FSH levels.
  - as women with anovulatory cycles have greater FSH:inhibin ratios compared with the ovulatory group.

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- Homozygous deletion of the inhibin α subunit gene (Inha) in mice.
  - The Inha -/- mice develop gonadal sex cord tumors within 6 weeks, causing death in males and females in 12 and 17 weeks
    - Males: sertoli cell tumors
    - Females: granulosa cell tumors
  - Indices of cachexia:
    - due to the lack of inhibins and the concomitant ↑ of activins; a 13- and 20-fold ↑ observed in the male and female
  - When the knockout mice were gonadectomized, life expectancy ↑
    - these mice developed adrenal tumors which caused death at 36 and 33 weeks in males and females

Introduction

- Several experiments demonstrate that it is inhibin which possesses the anti-tumourigenic properties.
  - The liver pathology and cachexia can be attributed to the over-expression of activins in the Inha -/- mouse, as this phenotype is also observed in mice given an excess of recombinant activin A.
  - The additional deletion of the activin type II receptor (ActRIIA) gene in the Inha -/- mice does not prevent tumor development but does prevent cachexia.

Introduction

- Several experiments demonstrate that it is inhibin which possesses the anti-tumourigenic properties.
  - Additionally the transplant of a wild-type ovary into the Inha -/- mouse demonstrated the ↓ of development of ovarian tumors.
    - local activin production is not sufficient for tumor development
    - the presence of circulating inhibin (from the transplanted wild-type ovary) is sufficient to stop tumor development

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- Over-expression of the inhibin α subunit gene in the rat also indicates that inhibin is required for normal ovarian function.
  - In rats with ↑ inhibins, there was a ↓ in litter size by 52% and a greater interval between pregnancies was observed.
  - Although no impairment in fertility was observed in males, in the females the ↓ in fertility was caused by a ↓ rate of folliculogenesis reflected by:
    - smaller population of antral follicles, corpora lutea
    - a 54% ↓ in the # of oocytes released/ovulated

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- Hence, the endocrine role of inhibin on the pituitary and its paracrine roles within the ovary support the concept that inhibin plays an important role in the regulation of ovarian function and folliculogenesis
  - it may be considered to be a potential candidate gene for the development of POF.

Introduction

- The hypothesis that in POF, a ↓ in circulating inhibin levels →
  - ↑ FSH concentrations
  - ↑ follicle recruitment
  - ↑ rate of follicle depletion
- Women with idiopathic POF have low serum levels of inhibin A and inhibin B, compared with age-matched fertile women.

Introduction

- Diagnosis is difficult and it is important to identify genes that impact ovarian function that can be linked to idiopathic POF.
  - Diagnose earlier with improved treatment

Methods

- MEDLINE search for all reports conducting population analysis of the incidence of the inhibin α subunit mutation (INHA G769A) in women with POF.

- 5 studies were included in the analysis of women with primary and secondary amenorrhea and controls.

Results - A genetic link between inhibin and POF

- A functional mutation in any 1 of the 3 inhibin genes could lead to a ↓ in the amount of bioactive inhibin.

  → This loss could remove the negative feedback on the pituitary → ↑ in FSH levels contributing to premature depletion of follicles → result in POF

Results - A genetic link between inhibin and POF

- The 1st evidence of a genetic link between inhibin and POF was established with the cytogenetic analysis of a POF patient who had a chromosomal translocation between chromosomes 2 and 15: 46,XX,t(2;15)(q32.3;q13.3).
  - As the inhibin α subunit locus is 2q33–36, it was important to determine whether inhibin might play a role in the development of POF.

Results - A genetic link between inhibin and POF

- Further investigation was aimed at mutational screening of the inhibin α subunit gene (INHA), focusing on the region encoding the inhibin mature peptide (αC), which after translation is proteolytically cleaved from the larger precursor peptide (ProαNαC).

- A missense mutation INHA G769A was identified causing an amino acid transition from alanine to a threonine.

Results – Incidence of the INH G769A in multi-ethnic populations

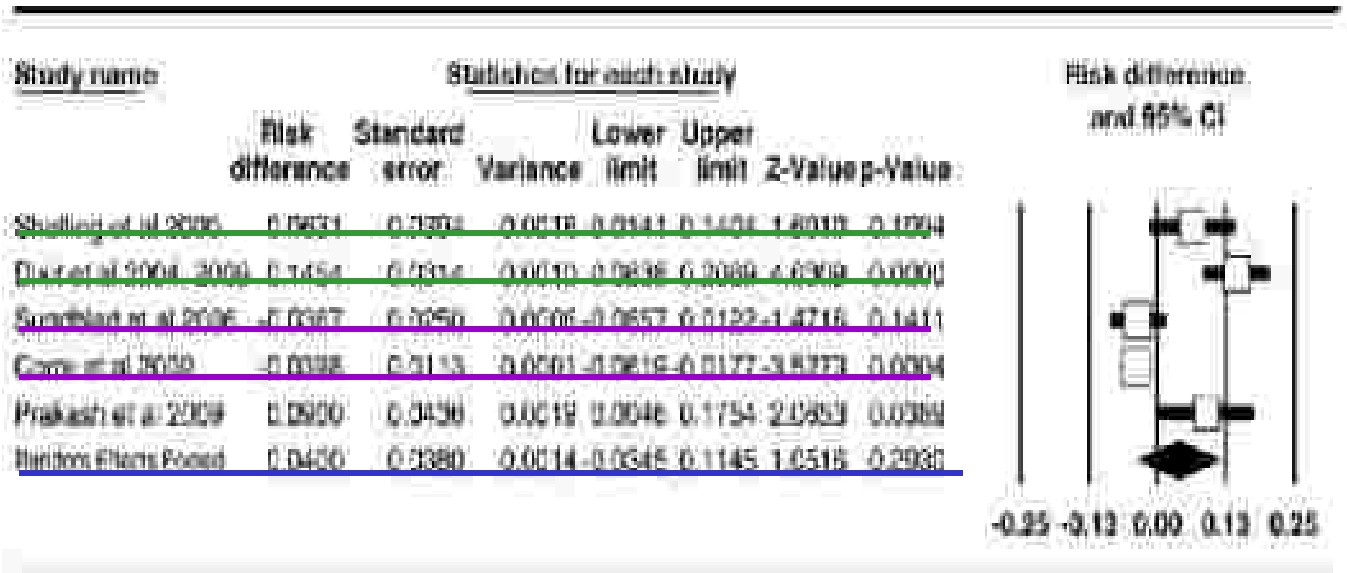
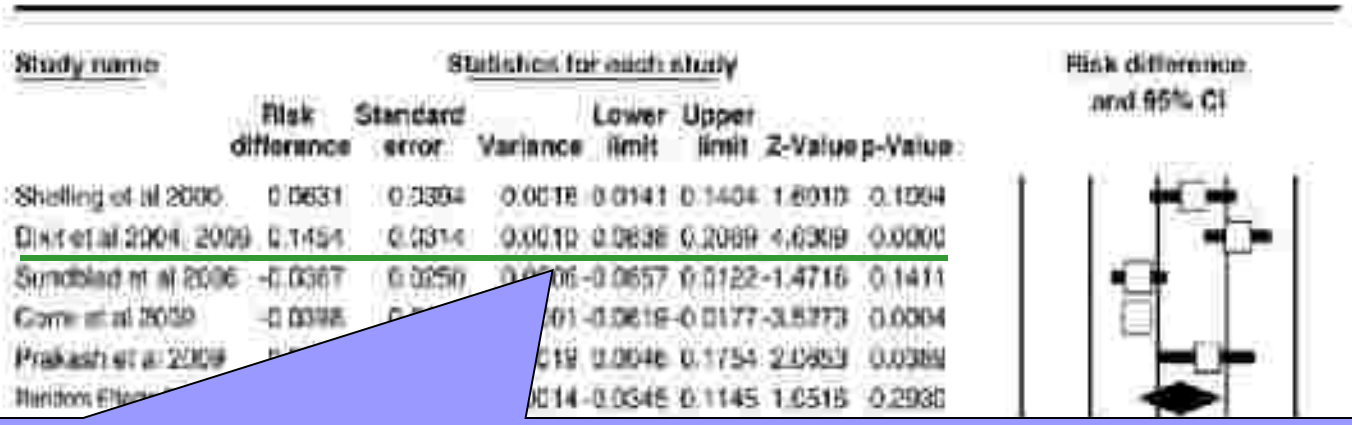


Figure 1 Meta-analysis of INH G769A gene variants in HDH and controls.

Random effects risk difference (RD) with 95% confidence interval (CI) for the risk of HDH in INH G769A carriers compared with controls in published studies, were calculated, using the Meta-Analysis V2 software. The RD of each study is marked with a square (□) and the CI displayed as a horizontal line. The pooled RD of all studies is represented with a black diamond (◆), its width marking the range of the 95% CI.



- 10.5% of the sporadic POF cases (n = 133) compared with 0.005% controls carried the INHA G769A mutation.
- 1 patient possessed a homozygous mutation
  - The circulating FSH levels in this patient were the highest recorded within the group studied (3 separate measurements of 100, 88 and 85 IU/l).
  - menopause at the age of 24 years

-Of the 60 women with primary amenorrhoea screened separately for the inhibin α subunit mutation, 6 were found to be carriers (10%).

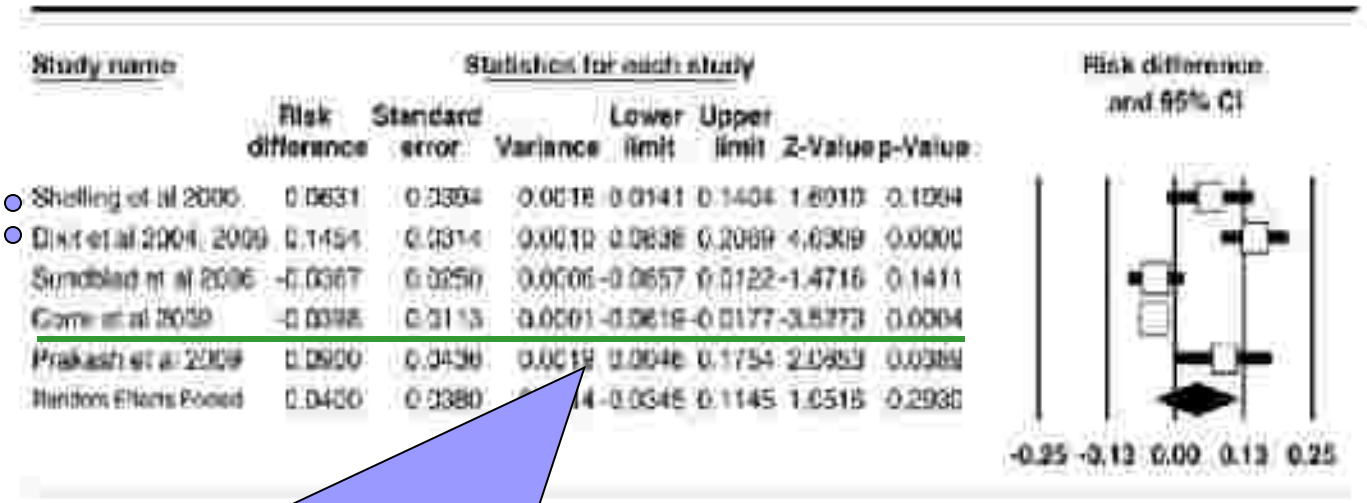


Figure 1 Meta-analysis of IN[illegible] and controls.

- The presence of the INHA G769A and an additional 3 novel new missense mutations
- The presence of the INHA G769A mutation was significantly greater in women with POF although 2 of the controls were also carriers.
- The presence of the INHA G769A mutation was associated with early onset of POF in this population. ◦

Results – Incidence of the INH G769A in multi-ethnic populations

- The initial study carried out in POF women in the Italian population showed a significant correlation between the INHA G769A mutation and POF (sporadic POF 4.5%, n = 157) and primary amenorrhoea (25%, n = 12) POF women.
  - significant link to the mutation in familial POF cases

  →a larger cohort of Italian and German subjects: no difference in mutation frequency was observed
- The INHA G769A mutation is rare in the Korean population where neither POF patients (n ¼ 84) or controls (n = 100) were identified as carriers.

Results – Incidence of the INH G769A in multi-ethnic populations

- Larger cohort studies with appropriate controls are required to further understand the relationship between the genetic variants in inhibin alpha subunit and POF.

- The current published studies indicate that in a susceptible population the INHA G769A mutation is associated with the early onset of POF.

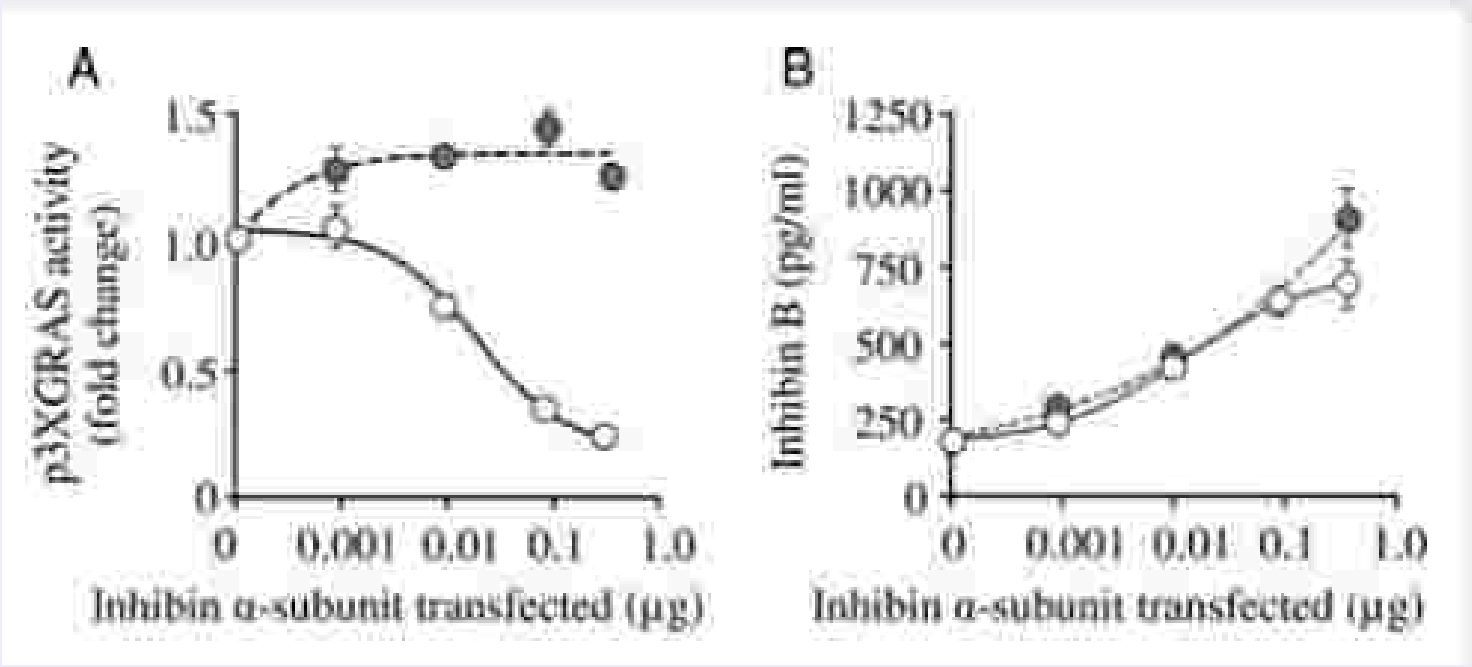


Figure 2 Difference in inhibin bioactivities between wild type and *INH G769A* (A257T) mutant inhibin B (data modified from Chand *et al.* (2007)).

Mouse gonadotroph LβT2 cells were transfected with increasing amounts of wild type or A257T inhibin α subunit expression vector and p3XGRAS-PRL-Luc reporter construct. Transfected cells were treated with 0.5 nM activin. (**A**) Effect of wild type or A257T inhibin on p3XGRAS-PRL-Luc activity, presented as fold change relative to basal reporter activity. (**B**) Dimeric inhibin B levels in conditioned culture media following overexpression of wild-type (○) or mutant (●) inhibin α subunits.

Results - Is there a physiological consequence of the INHA G769A mutation?

- As the INHA G769A mutation is commonly a heterozygous mutation → any consequence on inhibin biological function would not be a complete loss of function.
- It is suggested that in mutation carriers, a ↓ in inhibin function by 50%, would have effects at two stages:

  (i) fetal gonadal development

  (ii) regulation of normal folliculogenesis and ovulation

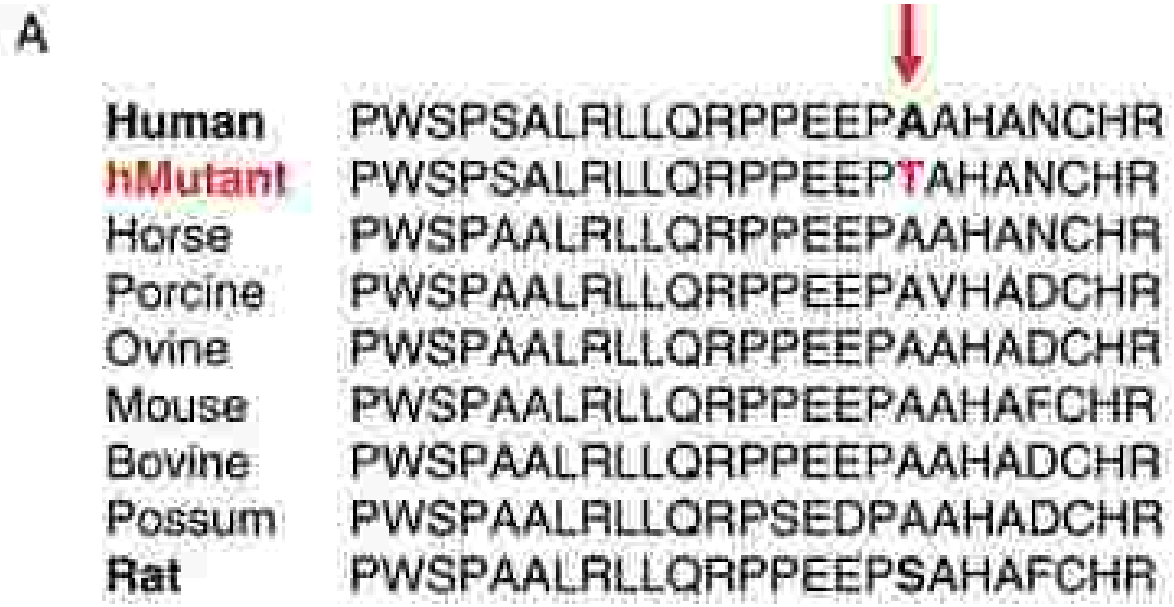
Results - Is there a physiological consequence of the INHA G769A mutation?

- A ↓ in inhibin biological potency could possibly hinder the normal development of the fetal ovary, continuing to affect ovarian development and function after birth.

- The ↑ of circulating FSH levels as a result of a reduction of the endocrine effect of inhibin may cause ovarian dysregulation, primarily at the follicular development stage.

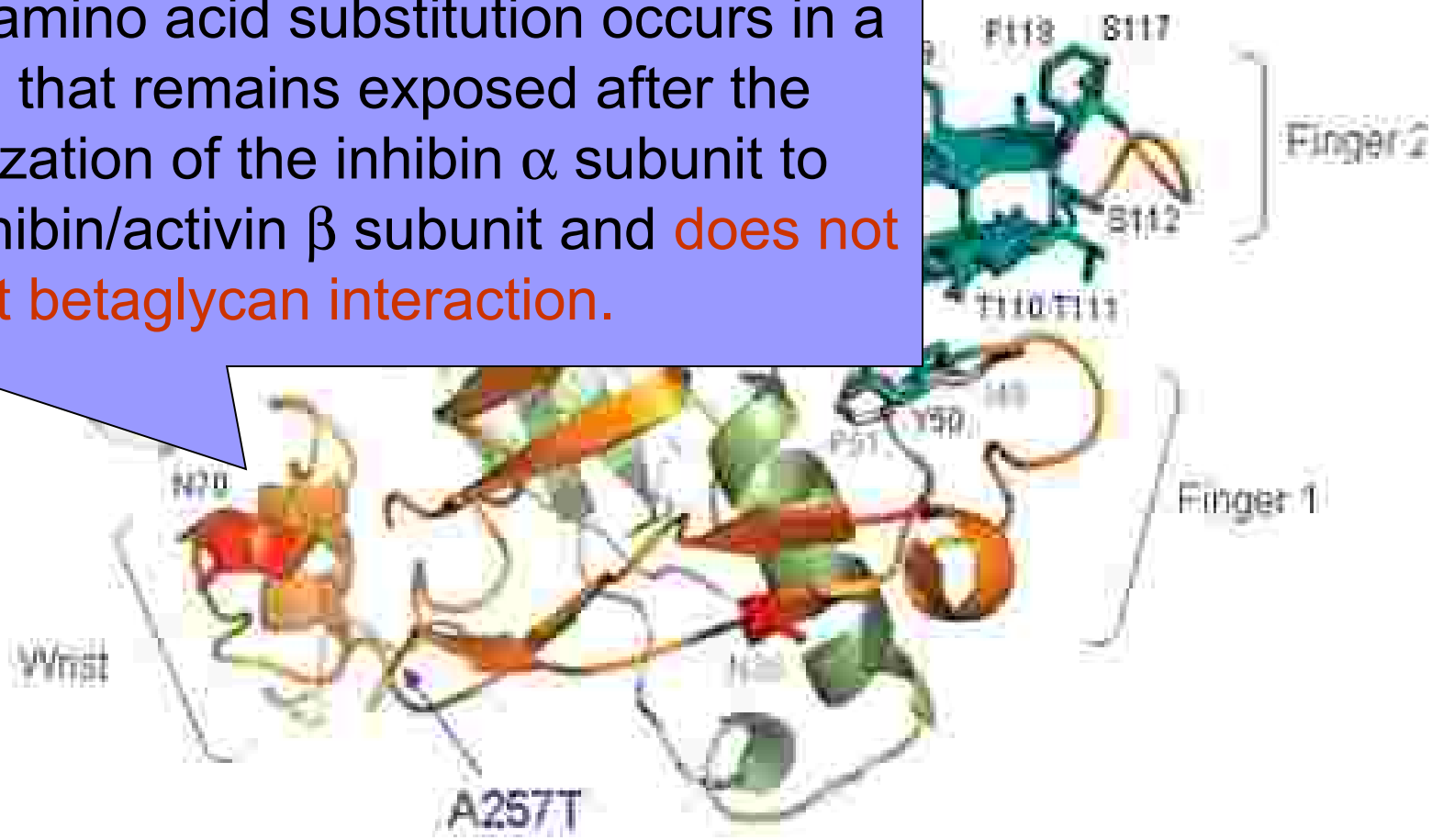
- As inhibin has important paracrine effects on the action of activin, GDF9 and BMP15 within the follicles, impairment in inhibin bioactivity could contribute towards aberrant folliculogenesis, maturation and atresia.

Results - Is there a physiological consequence of the INHA G769A mutation?



Results - Is there a physiological consequence of the INHA G769A mutation?

- this amino acid substitution occurs in a region that remains exposed after the dimerization of the inhibin α subunit to the inhibin/activin β subunit and does not impact betaglycan interaction.



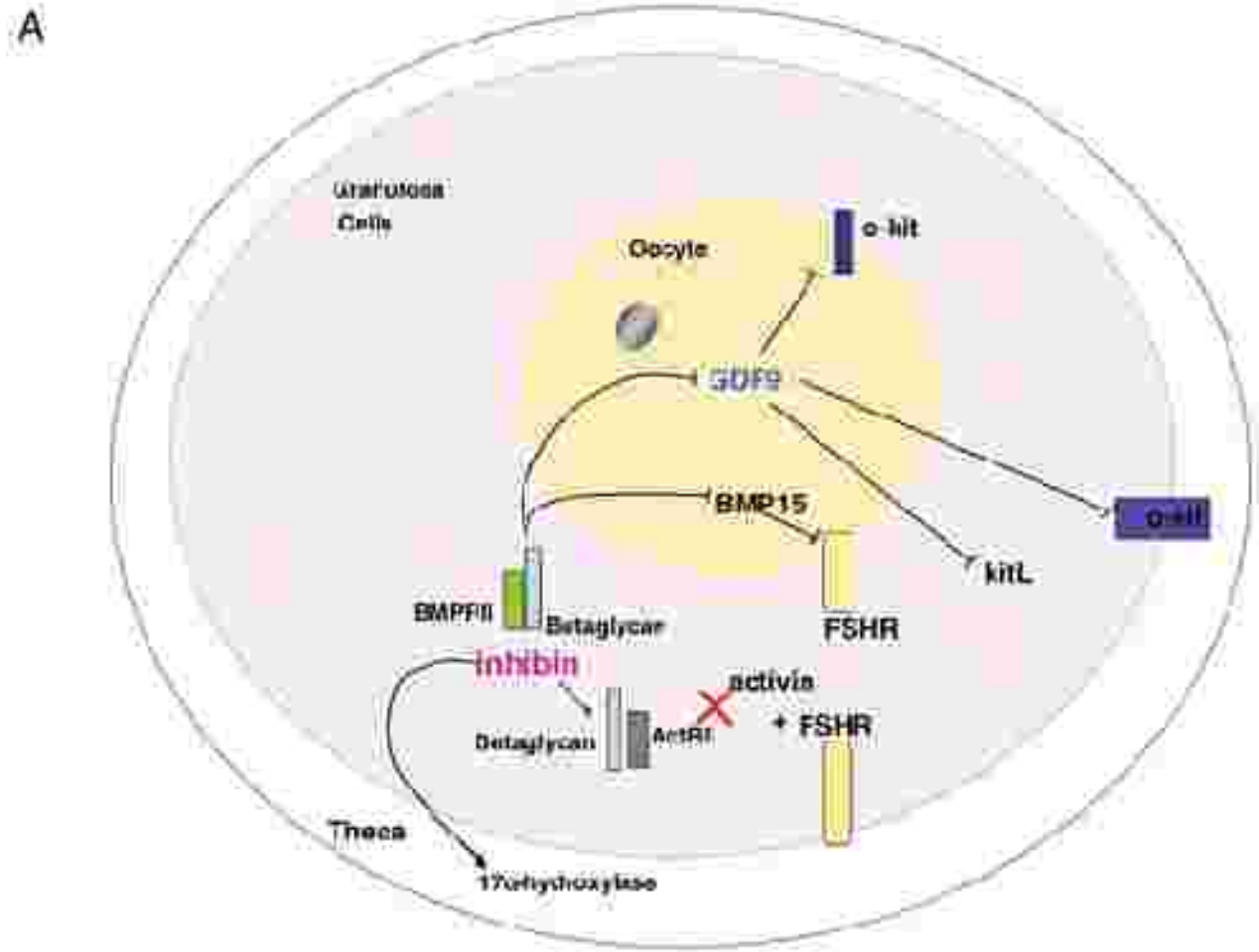
Results - Impact on the paracrine actions of inhibin

- The severity of the POF phenotype in mutation carriers could be compounded by the impairment of the paracrine actions of inhibin within the ovary:
  - Within ovarian theca-interstitial cells, inhibin stimulates LH-dependent androgen production → promoting follicular recruitment
  - steroidogenesis, possibly with the dysregulation of androgen production.
  - granulosa cell proliferation
  - the potentiating effects of activin and FSH in the ovary to ↑ FSH receptor expression.

Results - Impact on the paracrine actions of inhibin

- The action of inhibin as an activin antagonist is mediated via competition for the ActRIIs, facilitated by betaglycan.
- The betaglycan–inhibin complex binds to BMP receptor II (BMPRII) competing for BMP binding.
  - In this manner inhibin could antagonize the paracrine actions of BMPs, a subgroup of growth factors and cytokines from the TGFb superfamily.

Results - Impact on the paracrine actions of inhibin



Results - Impact on the paracrine actions of inhibin

- A BMP essential for follicle development to the secondary stages is BMP15 (also known as Growth Differentiation Factor 9B).
  - The earliest role: the initiation of granulosa cell proliferation in primary follicles.
  - enhances estrogen and progesterone synthesis with the stimulation of aromatase expression and inhibits the premature luteinisation of large follicles.
  - in large antral and Graafian follicles causes the down-regulation of FSHR expression → maintaining a control on progesterone production until ovulation
  - stimulates granulosa cell secretion of Kit Ligand which bind to its receptor c-kit in the oocyte
    - Kit Ligand actions cause negative feedback on BMP15-dependent granulosa cell proliferation

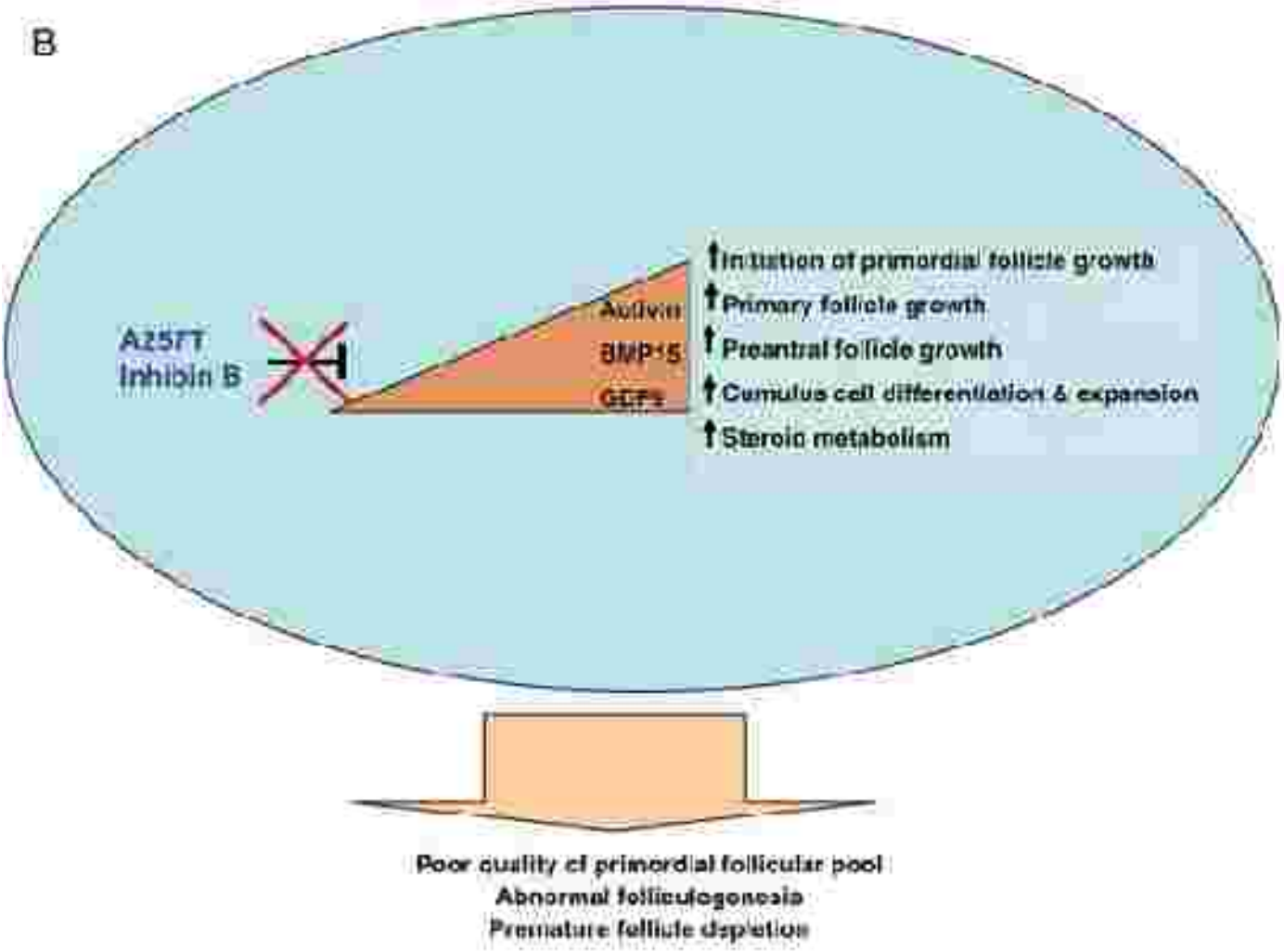
Results - Impact on the paracrine actions of inhibin

- Another TGFb ligand that signals via BMPRII is GDF9.
  - expressed in follicles in the primary stage and onwards
  - granulosa cell proliferation, differentiation and cumulus expansion, with the induction of hyaluronan synthase 2 (HAS2), cyclooxygenase 2 (COX2), and steroidogenic acute regulator protein (StAR).
  - rare mutations in this gene are linked to POF

Results - Impact on the paracrine actions of inhibin

- The phenotype of a double knockout transgenic mouse, in which the genes encoding inhibin a subunit and GDF9 have been deleted (Inha -/-GDF9-/-)
  - the presence of healthy follicles at all stages of folliculogenesis, until the late pre-antral stage, after which there is formation of granulosa cell tumors.

Results - Impact on the paracrine actions of inhibin



Results - Phenotypic heterogeneity among INHA G769A mutation carriers

- Genetic studies are difficult with infertility disorders due to the inability to ascertain large families over multiple generations, as by definition, infertility within a family will lead to small families.
  - Using a candidate gene approach many genes have now been identified as causes of idiopathic POF, such as FSHR, FOXL2, NOBOX and FMR1.

Results - Phenotypic heterogeneity among INHA G769A mutation carriers

- The prevalence of unaffected carriers could be a relatively common occurrence in families with a history of POF.
  - higher frequency in the Italian population
  - the Indian population is most profoundly affected by the INHA G769A mutation
  - genetic variability is confounded by ethnic-dependent factors?

Results - Phenotypic heterogeneity among INHA G769A mutation carriers

- The age of onset of POF in carriers of the mutation varies among different population groups.
  - the age of onset of menopause in the New Zealand and Indian population is considerably lower (13.6 years earlier) compared with that of Italian women who carried the INHA G769A mutation.
  - The incidence of menopause before the age 25 years in the New Zealand and Indian carrier population was 100 and 90%
    - Italian POF population: none of the carriers experienced menopause before the age of 30 years.
- In POF patients who were carriers of the INHA G769A mutation, approximately 30% experienced primary amenorrhoea.

Results - Can phenotypic heterogeneity among INHA G769A mutation carriers be related to ethnic-dependent factors?

- A recent analysis of pregnancy outcomes in polycystic ovarian syndrome (PCOS) patients using IVF techniques supports variability between South Asian (Indian) and Caucasian populations.
  - Caucasian PCOS patients had a 2.5 x higher chance of ongoing pregnancy compared with South Asian PCOS patients.
    - ↑ sensitivity among South Asians to gonadotrophin stimulation which resulted in a greater change in FSH levels

Results - Can phenotypic heterogeneity among INHA G769A mutation carriers be related to ethnic-dependent factors?

- A ↓ in inhibin bioactivity would cause ↑ secretion of FSH; and in a susceptible population → a small variability in FSH levels but a heightened sensitivity to gonadotrophin stimulation would explain the earlier onset of POF observed in this population group.
  - A subtle change in FSH levels in a hypersensitive system could account for increased follicular loss, likely through aberrant folliculogenesis and increased atresia.

Conclusion

- POF is a common condition and is of growing concern to the general population due to the increasing trend of delaying pregnancy.
- Diagnosis is often delayed due to an extensive variability in symptoms and no predicting factors for idiopathic POF.
- The issues regarding personal health and quality of life of the affected women with the occurrence of menopause at a young age is also of concern.
  - Hence there is a clear need for improved diagnostic screening before the onset of disease.

Conclusion

- The identification of an autosomal mutation in the inhibin α subunit gene (INHA G769A) that is significantly linked to POF is of benefit in terms of improving our understanding of the role of inhibin in the regulation of ovarian biology and fertility.

- Although the reduction of inhibin B bioactivity by the INHA G769A mutation is clearly not the only cause for the condition, evidence suggests that this change may serve as a susceptibility factor, increasing the likelihood of POF.

Conclusion

- Other predisposing factors including other genes, ethnicity and lifestyle factors will likely play a role, but at the moment, the roles of these factors remain largely unknown.

- Furthermore these studies contribute to the overall understanding of oligogenetic diseases such as POF.